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European Human Genetics  
Conference 2009

May 23 – 26, 2009  
Vienna, Austria

Abstracts



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**EUROPEAN  
HUMAN GENETICS  
CONFERENCE 2009**

**Austria Center Vienna**

**Vienna, Austria**

**Saturday, May 23 – Tuesday, May 26, 2009**

**Abstracts**

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### Future European Human Genetics Conferences

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European Human Genetics Conference 2010  
June 12 – 15, 2010  
Gothenburg, Sweden

European Human Genetics Conference 2011  
May 28 - 31, 2011  
Amsterdam, The Netherlands

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**PL1.1****Genetic epidemiology of lipid-associated disorders****F. Kronenberg:**

Division of Genetic Epidemiology; Department of Medical Genetics, Molecular and Clinical Pharmacology; Innsbruck Medical University, Innsbruck, Austria. Lipids are essential for many biological processes such as the building of membranes, energy substrates, hormones and biological signals. A well-controlled balance of lipid demand and supply under changing nutritional conditions with storage in various cells in form of lipid droplets is therefore important. The Austrian Genome Project "GOLD" (Genomics of Lipid-associated Disorders) aims to discover genes, gene products and metabolites required for the generation, structural integrity and catabolism of lipid droplets. Genetic epidemiology is one of thirteen research teams of this multidisciplinary consortium and aims to identify genetic variation of genes involved in these processes by two approaches: an hypothesis-free approach with genome-wide association studies on quantitative lipids traits and a hypothesis-driven approach with association studies on genes which might be involved in lipid-related phenotypes. The genome-wide association studies revealed not only yet unknown gene regions influencing lipid levels but also demonstrated that regions far upstream or downstream of the gene have a strong impact on lipid levels. In the hypothesis-driven approach genetic variation within the newly discovered adipose triglyceride lipase (ATGL) which hydrolyzes the first ester bond of triglycerides was found to have a strong impact on free fatty acid and triglyceride concentrations. Furthermore, ATGL was found to have a large number of rare and very rare variants which are subject of investigation. Finally, adiponutrin, a protein structurally related to ATGL was recently identified to be associated with hepatic fat content and liver enzyme levels. Using five different studies we clearly demonstrated a strong impact of variants within adiponutrin with total and non-HDL cholesterol levels.

**PL1.2****Clinical significance of embryoscopy for early intrauterine deaths**T. Philipp<sup>1</sup>, E.R. Separovic<sup>2</sup>, S. Sladek<sup>3</sup>, P. Hofmeister<sup>3</sup>, A. Reiner<sup>3</sup>;<sup>1</sup> Department of Obstetrics and Gynecology, Danube Hospital, Vienna, Austria,<sup>2</sup> Cytogenetic Laboratory, Department of Pathology, B. C. Children's Hospital, Vancouver B.C., Canada, <sup>3</sup> Department of Pathology, Cytogenetic Laboratory, Danube Hospital, Vienna, Austria.

Introduction: About 20 % of clinically recognized pregnancies are aborted, the majority of these being early spontaneous pregnancy losses before twelve gestational weeks. Pathological examination of first trimester losses is difficult because the embryo/fetus is only rarely retrieved unaffected by the damage caused by either instrumental evacuation or spontaneous passage. Transcervical embryoscopy prior to dilatation and curettage in cases of missed abortion enabled us to study the external features of the embryo detected in first trimester missed abortions. The importance of accurate descriptions of the dead embryo/fetus to answer specific questions of parents as to the probable cause of death and the future risk of recurrence of abortion or an abnormal infant is discussed.

Material and Method: Prior instrumental evacuation of the uterus a rigid hysteroscope (12-degree angle of view with both biopsy and irrigation working channel, Circon Ch 25-8 mm) was inserted transcervically into the uterine cavity. The amnion was opened with microscissors (CH 7-2mm) to obtain a detailed view of the embryo. After evacuation of the uterus chorionic villi were analysed cytogenetically, using either standard G-banding cytogenetic techniques or comparative genomic hybridization in combination with flow cytometry.

Results: The embryo or early fetus was visualized in over 800 first trimester missed abortions in this ongoing study and a high rate of developmental defects could be found.

Localized developmental defects, usually represented by the combination of several external developmental defects included neural tube defects (spina bifida, encephalocele, anencephalus), holoprosencephaly, microcephaly, facial dysplasia, various forms of cleft lip, fused mouth, limb abnormalities (syndactyly, polydactyly, cleft hand, limb reduction defect), gastroschisis and omphalocele, amniotic adhesions and duplication anomalies (thoracophagus, acardius anceps).

Chromosomal abnormalities included mostly numerical aberrations such as monosomy X, trisomy of chromosomes; triploidy; tetraploidy. Trisomies for all chromosomes with the exception of chromosomes 1

were observed.

The highest rate of chromosome abnormality was observed in the category of embryos showing combined (>2) localized defects, and the lowest in morphologically normal embryos.

Conclusions: The detection of a specific defect in an embryo with normal chromosomes can be essential. It will confront investigators with factors usually not considered to be etiologically related to early pregnancy loss. Our data indicate that malformations of first-trimester intrauterine deaths with normal chromosomes are etiologically heterogeneous. An accurate description of these specimens is essential as it can help to identify the specific mechanism leading to the observed developmental defects and might assist investigators in answering specific questions from parents concerning the probable cause of death and the risk of recurrence in a subsequent pregnancy.

**PL1.3****Immunology****J. Penninger:**

IMBA - Institute of Molecular Biotechnology GmbH, Vienna, Austria.

No abstract received as per date of printing. Please see [www.eshg.org/eshg2009](http://www.eshg.org/eshg2009) for updates.

**PL2.1****Genetics meets metabolomics: a genome-wide association study of metabolite profiles in human serum**

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The rapidly evolving field of metabolomics aims at a comprehensive measurement of ideally all endogenous metabolites in a cell or body fluid. It thereby provides a functional readout of the physiological state of the human body. Genetic variants that associate with changes in the homeostasis of key lipids, carbohydrates or amino acids are not only expected to display much larger effect sizes due to their direct involvement in metabolite conversion/modification, but should also provide access to the biochemical context of such variations, in particular when enzyme coding genes are concerned.

To test this hypothesis we conducted the first GWAS with metabolomics, based on the quantitative measurement of 363 metabolites in serum of 284 male participants of the KORA study. We found associations of frequent single nucleotide polymorphisms with considerable differences in the metabolic homeostasis of the human body, explaining up to 12% of the observed variance. Using ratios of certain metabolite concentrations as a proxy for enzymatic activity, up to 28% of the variance can be explained (p-values 10<sup>-16</sup> to 10<sup>-21</sup>). We identified four genetic variants in genes coding for enzymes (FADS1, LIPC, SCAD, MCAD) where the corresponding metabolic phenotype (metabotype) matches the biochemical pathways in which these enzymes are active.

Our results suggest that common genetic polymorphisms induce major differentiations in the metabolic make-up of the human population. These genetically determined metabotypes may subscribe the risk for a certain medical phenotype, the response to a given drug treatment, or the reaction to a nutritional intervention or environmental challenge.

**PL2.2****Massive parallel sequencing of ataxia genes after array-based enrichment**

A. Hoischen<sup>1</sup>, C. Gilissen<sup>1</sup>, W. van der Vliet<sup>1</sup>, P. Arts<sup>1</sup>, N. Wieskamp<sup>1</sup>, S. Vermeer<sup>1</sup>, R. Meijer<sup>1</sup>, M. Buckley<sup>1</sup>, B. Kremer<sup>2</sup>, N. van Slobbe-Knoers<sup>1</sup>, J. Veltman<sup>1</sup>, H. Scheffer<sup>1</sup>;

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Targeting multiple disease genes by massively parallel sequencing has tremendous diagnostic potential but requires new 'front-end' methods to enrich templates to be sequenced. Here we validated the array-based sequence capture method for medical sequencing approaches in heterogeneous genetic disorders. As a model disease we chose autosomal recessive ataxia and selected 4 patients with known mutations in ataxia genes. Genomic sequences of all known disease genes, including all intronic sequences and 5kb up- and downstream of each gene, as well as a novel ataxia candidate locus were represented on a single oligonucleotide array, comprising 2.5 Mb of genomic sequence in total. After enrichment each of the patients DNA was analyzed by one quarter of a Roche GS FLX Titanium sequencing run, resulting in approximately 100 Mb of genomic sequence per patient. This was sufficient to reach an average per base coverage of 34-fold in all targeted regions. Enrichment showed high specificity, as up to 88% of all reads mapped uniquely to the targeted regions. Very few single reads mapped to non-targeted regions, interestingly mainly to chr.9\_random, which might indicate that this region represents a minor allele in the existing human genome assembly. Importantly, this approach enabled an unbiased detection of all known mutations in our 4 patients, deletions and point mutations, as well as known SNP variants (hetero- and homozygous). These results show that massive parallel sequencing of enriched samples enables tailor-made genetic diagnosis of heterogeneous disorders.

**PL2.3****The SLC29A3 gene is mutated in Pigmented Hypertrichosis with Insulin Dependent Diabetes Mellitus syndrome and interacts with the insulin signalling pathway**

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Pigmented hypertrichotic dermatosis with insulin dependent diabetes (PHID) syndrome is a recently described autosomal recessive disorder associated with predominantly antibody negative, insulin-dependent diabetes mellitus. In order to identify the genetic basis of PHID and study its relationship with glucose metabolism we performed homozygosity mapping in five unrelated families followed by candidate gene sequencing. Five loss of function mutations were identified in the SLC29A3 gene which encodes a member of a highly conserved protein family that transports nucleosides, nucleobases and nucleoside analogue drugs, hENT3. We show that PHID is allelic with a related syndrome without diabetes mellitus, H syndrome. The interaction of SLC29A3 gene with insulin signaling pathways was then studied using an established model in *Drosophila melanogaster*. Ubiquitous knockdown of the *Drosophila* ortholog of hENT3, dENT1 is lethal under stringent conditions whereas milder knockdown induced scutellar bristle phenotypes similar to those previously reported in knockdown of the *Drosophila* ortholog of the *Islet* gene. A cellular growth assay showed a reduction of cell size/number which could be rescued or enhanced by manipulation of the *Drosophila* insulin receptor and its downstream signaling effectors, dPI3K and dAkt. In summary, inactivating mutations in SLC29A3 cause a syndromic form of insulin dependent diabetes in humans and in *Drosophila* profoundly affect cell size/number through interactions with the insulin signaling pathway. These data suggest that the further investigation of the role of SLC29A3 in glucose metabolism is a priority for diabetes research.

**PL2.4****Duplication of conserved non-coding sequence elements****- a novel mechanism in the pathogenesis of congenital malformations**

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Two thirds of the sequence conserved among mammals is not protein coding. The precise function of such non-coding sequence elements (CNEs) is unknown but they have been proposed to regulate time and tissue specific gene expression over distances as great as 1Mb. Using high-resolution array CGH we detected CNE-containing duplications in patients with congenital malformations. We identified duplications of 1) a regulatory sequence 1Mb upstream of *SHH* (ZRS) in triphalangeal thumb-polysyndactyly syndrome and in 2) Laurin-Sandrow syndrome, 3) a 5kb regulatory element 110kb downstream of *BMP2* in brachydactyly type A2, 4) 2Mb containing several CNEs upstream of *SOX9* in Cooks syndrome, and 5) 1.7Mb 5' of *MSX2* in a condition resembling cleidocranial dysplasia. In all cases the duplications are arranged in tandem. We show that the CNE contained in the duplication at the *BMP2* locus is an enhancer regulating *BMP2* expression exclusively in the limbs, thus, functioning as *cis*-regulatory element. We postulate that duplications of *cis*-regulatory elements cause selective deregulation of the target gene resulting in disturbance of dosage-dependent signalling pathways only in those areas and/or time points that correspond to the CNE's regulatory potential. Our data provide the molecular cause for so far genetically unresolved conditions (Laurin-Sandrow, Cooks syndrome) and show genetic heterogeneity for others. Furthermore, duplications of CNEs can be considered a novel genetic mechanism for developmental defects. Given the importance of temporal-spatial gene regulation during embryonic development it is to be expected that a large number of developmental defects are caused by mutations affecting such distant enhancers/repressors.

**PL2.5****Mitosis updated - PICH and the anaphase threads**

T. Schwarzbraun<sup>1</sup>, L. Wang<sup>2</sup>, P. Ulz<sup>1</sup>, E. A. Nigg<sup>2</sup>, M. R. Speicher<sup>1</sup>;

<sup>1</sup>Institute of Human Genetics, Graz, Austria, <sup>2</sup>Max-Planck Institute of Biochemistry, Martinsried, Germany.

The process of mitosis has been illustrated in more or less unchanged ways in textbooks for decades and still resembles the original observations made by Flemming back in 1882. However, PICH (Plk1-interacting checkpoint helicase) was recently identified as an essential component of the spindle assembly checkpoint and shown to localize to kinetochores, inner centromeres, and most interestingly, it decorates thin threads connecting separating sister-chromatids even until late anaphase. PICH-positive threads evolve from inner centromeres as they stretch in response to tension reaching up to 15  $\mu$ m in length. With the discovery of DNA threads connecting sister-chromatids until late anaphase a new level of cell cycle regulation seems likely. The properties of the PICH protein lead to the hypothesis that it associates with catenated centromeric DNA, where it may act as a tension sensor to monitor the bipolar attachment of sister kinetochores and thus ensuring accurate chromosome segregation. Indeed, we could recently demonstrate that these threads are in fact mainly constituted of stretched alploid centromeric DNA and that topoisomerase activity is required during anaphase for the resolution of PICH-positive threads. Additionally, knock-down as well as over-expression of the PICH protein result in severe chromosomal mis-segregation. These data indicate that the complete separation of sister chromatids occurs later than previously assumed as well as that PICH and the alploid centromeric DNA repeats are part of an additional mechanism to safeguard the genomic integrity.

**PL2.6****Genome-wide association reveals master eQTL regulators of microRNA expression variation in human fibroblasts**

**C. Borel, S. Deutsch, A. Letourneau, M. Gagnebin, C. Gehrig, E. Falconnet, Y. Dupré, S. E. Antonarakis;**

*Department of Genetic Medicine and Development, University of Geneva Medical School, CH, Geneva, Switzerland.*

MicroRNAs (miRNA) have emerged as new class of regulatory non-coding RNAs modulating the processing of over a third of human transcripts. Inter-individual variation of miRNA levels is likely to influence expression of their target genes, and may therefore contribute to phenotypic differences, including susceptibility to common disorders. Nothing is known at present regarding the genetic control of microRNA expression levels. In this study, we have performed a whole-genome, quantitative association study of 365 microRNA expression phenotypes in 180 primary fibroblasts from Caucasian newborns of the GenCord project, genotyped with the Illumina HumanHap550 array. We find extensive variation in microRNA expression levels and estimate that 33% of microRNAs are differentially expressed. For 121 selected expressed microRNAs, GWAS yielded highly significant *cis*- (9%) and *trans*- (11%) associations. Furthermore, we characterized genomic regions containing master regulators that influence the expression of multiple miRNAs, thus providing a previously unknown mechanism suggesting co-regulation of miRNA expression.

This is the first attempt to characterize the genetic regulation of miRNA expression levels. Loci identified through this approach are likely to be important determinants of human phenotypes.

C.B and S.D contributed equally to this work.

**PL3.1****Weird animal genomes, sex and dumb design**

**J. Graves;**

*The University of Melbourne, Department of Zoology, Melbourne, Australia.*

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**PL3.2****Genetics and sociobiology of microbes**

**K. Foster;**

*Center for Systems Biology, Harvard University, Cambridge, MA, United States.*

*"If it could be proved that any part of the structure of any one species had been formed for the exclusive good of another species, it would annihilate my theory, for such could not have been produced through natural selection."* Darwin (1859)

Since Darwin, evolutionary biologists have been troubled by cooperative behavior. Why do organisms frequently evolve social behaviors that promote others at an apparent cost to their own reproduction?

For example, honeybee workers labor their whole life without reproducing, birds make alarm calls, and humans often help one another. This fundamental question has received considerable attention over the last 50 years with the development of the field of sociobiology. This has resulted in a solid base of theory, centered on principles like inclusive-fitness, and a myriad of empirical tests. It is now widely accepted that cooperative behaviors evolve because they directly help the actor alongside any recipients, or they help individuals who share more alleles with the actor than predicted by chance (genetic relatedness), or both. One major group that remains relatively unexplored, however, is the microbes, whose full spectrum of sociality only recently came to light. My group studies how social environment and relatedness affects microbial behavior in a number of model systems, including biofilm-forming bacteria, slime molds and budding yeast. We find that microbes are extremely sensitive to social context - both in real time and over evolutionary time - and use them to better understand the genetic and genomics of social traits; a pursuit that is difficult in the more classical model organisms for social behavior.

**PL3.3****Language as Kluge**

**G. Marcus;**

*New York University, NY, United States.*

In fields ranging from reasoning to linguistics, the idea of humans as perfect, rational, optimal creatures is making a comeback – but should it be? Hamlet's musings that the mind was "noble in reason ... infinite in faculty" have their counterparts in recent scholarly claims that the mind consists of an "accumulation of superlatively well- engineered designs" shaped by the process of natural selection (Tooby and Cosmides, 1995), and the 2006 suggestions of Bayesian cognitive scientists Chater, Tenenbaum and Yuille that "it seems increasingly plausible that human cognition may be explicable in rational probabilistic terms and that, in core domains, human cognition approaches an optimal level of performance", as well as in Chomsky's recent suggestions that language is close "to what some super-engineer would construct, given the conditions that the language faculty must satisfy".

In this talk, I will argue that this resurgent enthusiasm for rationality (in cognition) and optimality (in language) is misplaced, and that the assumption that evolution tends creatures towards "superlative adaptation" ought to be considerably tempered by recognition of what Stephen Jay Gould called "remnants of history", or what I call evolutionary inertia. The thrust of my argument is that the mind in general, and language in particular, might be better seen as what engineers call a kluge: clumsy and inelegant, yet remarkably effective.

**S01.1****Genome variation, gene regulation, and human disease****S. McCarrol;***Broad Institute, Program in Medical and Population Genetics, Cambridge, MA, United States.*No abstract received as per date of printing. Please see [www.eshg.org/eshg2009](http://www.eshg.org/eshg2009) for updates.**S01.2****New methods for detecting rare variants associations****A. Kong;***deCODE genetics, Reykjavik, Iceland.*No abstract received as per date of printing. Please see [www.eshg.org/eshg2009](http://www.eshg.org/eshg2009) for updates.**S01.3****Rare and common variants in human disease****D. Goldstein;***Duke University, Center for Human Genome Variation, Durham, NC, United States.*

There are now several confirmed common variants that influence common diseases, responses to infection, and responses to drugs. For most common diseases however all common variants identified explain only a few percent of the known heritability, and many of the signals emerging from genome wide association studies have yet to be tracked to single common variants, raising the possibility that in some cases the signals emerge from associated sets of rare variants. Here I argue that progress in identifying the so called 'missing heritability' for many human traits will be facilitated using an extreme phenotype whole-genome sequencing paradigm. I illustrate the basic structure and motivation for this approach using examples from our work on host determinants of control of HIV-1

**S02.1****Non-coding antisense RNAs epigenetically regulate transcription in human cells****K. V. Morris;***Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA, United States.*

Small RNA targeting of promoters in human cells has been shown to modulate transcriptional gene silencing. While the mechanism involved in transcriptional gene silencing has been shown to require Argonaute 1 (Ago-1), Histone Deacetylase 1 (HDAC-1), and DNA methyltransferase 3a (DNMT3a), the endogenous RNA trigger directing these proteins to targeted promoters in human cells had remained unknown. We present evidence here suggesting that non-coding antisense RNAs function in human cells as effector molecules driving transcriptional gene silencing. The antisense non-coding RNAs guide epigenetic remodeling complexes to target promoters in an Ago-1 mediated manner. When these regulatory antisense non-coding RNAs are degraded using RNA interference there is a concomitant activation of the sense/mRNA promoter which is under the regulatory control of the particular antisense RNA. The data presented here suggests that in human cells, bidirectional transcription is an endogenous gene regulatory mechanism whereby antisense non-coding RNAs direct epigenetic regulatory complexes to the corresponding sense promoters, resulting in RNA directed epigenetic gene regulation. These observations support the notion that epigenetic silencing of tumor suppressor genes may be the result of an imbalance in bidirectional transcription levels. This imbalance allows the unchecked antisense RNA to direct silent state epigenetic marks to the sense promoter, resulting in stable transcriptional gene silencing.

**S02.2****The role of microRNAs in brain tumors****D. Beier<sup>1</sup>, J. Y. Zhu<sup>2</sup>, A. Eichner<sup>2</sup>, C. Beier<sup>1</sup>, G. Meister<sup>2</sup>;**<sup>1</sup>*Neurologische Universitätsklinik im Bezirksklinikum, Regensburg, Germany,*<sup>2</sup>*Center for integrated protein Sciences Munich (CIPSM), Laboratory of RNA Biology, Max-Planck-Institute of Biochemistry, Martinsried, Germany.*

MicroRNAs (miRNAs) are fundamental regulators of gene expression that direct processes as diverse as cell metabolism, lineage specification or cell differentiation. Consistently, miRNAs are frequently misexpressed in cancer. In several cancer types including breast cancer

and glioblastoma, a minor cell population with stem cell-like properties has been identified and these cells have been termed cancer stem cells. Here we report the miRNA expression profile of glioblastoma stem cells. We find that both miR-9 as well as its corresponding miR-9\* are highly abundant in cancer stem cell-containing cell populations. Inhibition of both miRNAs leads to reduced tumor growth *in vitro* and in nude mice. We further find that inhibition of miR-9/9\* leads to enhanced neuronal differentiation and therefore miR-9/9\* inhibit differentiation of glioblastoma stem cells and maintain their 'stemness'. Since tumor stem cells are difficult to target and very often survive therapy, our findings could be the basis for novel therapeutic strategies.

**S02.3****mRNA splicing and disease****U. Fischer;***Department of Biochemistry at the Biocentre, University of Würzburg, Würzburg, Germany.*

Mutations that affect pre-mRNA processing are the cause for many genetic diseases. Most such mutations target *cis*-acting regulatory sequences in a given transcript, thus preventing its proper maturation. Only recently however, mutations in *trans*-acting factors involved in pre-mRNA processing have likewise been linked to disease. One prominent example is spinal muscular atrophy (SMA) a monogenic, neuromuscular disorder caused by reduced levels of functional survival motor neuron (SMN) protein. This ubiquitous factor is part of a complex that mediates the formation of spliceosomal snRNPs. The detailed biochemical investigation of SMN under normal conditions and in SMA has provided clues of how mutations in factors with general functions elicit tissue specific phenotypes.

**S03.1****Predictive Genetic Testing for Cardiovascular Diseases: Impact on Carrier Children.****E. M. A. Smets<sup>1</sup>, T. M. Meulenkamp<sup>1</sup>, M. M. H. Stam<sup>1</sup>, A. Tibben<sup>2</sup>, E. D. Mollema<sup>3</sup>, I. M. van Langen<sup>3</sup>, A. Wiegman<sup>4</sup>, G. M. de Wert<sup>5</sup>, I. D. de Beaufort<sup>6</sup>, A. A. M. Wilde<sup>7</sup>;**<sup>1</sup>*Medical Psychology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, <sup>2</sup>Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands, <sup>3</sup>Clinical Genetics, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, <sup>4</sup>Pediatric Lipid Clinic, Emma's Children Hospital, Academic Medical Center/University of Amsterdam, Amsterdam, The Netherlands, <sup>5</sup>Health Ethics and Philosophy, Faculty of Medicine, Maastricht University, Maastricht, The Netherlands, <sup>6</sup>Health care ethics at the Erasmus Medical Center of Erasmus University, Rotterdam, The Netherlands, <sup>7</sup>Cardiology, Academic Medical Center/University of Amsterdam, Amsterdam, The Netherlands.*

Familial Hypercholesterolemia, Hypertrophic Cardiomyopathy and Long QT Syndrome are genetic cardiovascular conditions which may lead to sudden cardiac death at a young age. Preventive measures include lifestyle modifications, medications and/or cardiac devices. Identification of carrier children can protect them for the potentially life threatening consequences at a young age, but may at the same time have negative consequences.

Our study aimed to explore a) the manner in which children perceive their carrier status, b) the impact on their daily lives and c) the strategies used to cope with these consequences and d) how their quality of life compares to the quality of life of other children their age.

Children (aged 8-18) who tested positive for LQTS (n=11), HCM (n=6) or FH (n=16) and their parents participated in semi-structured audiotaped interviews. Children's health-related quality of life was assessed with a children and parent version self-report questionnaire.

The children were overall quite articulate about the disease they were tested for, including its mode of inheritance. They expressed positive future health perceptions, but feelings of controllability varied. Adherence and side-effects were significant themes with regard to medication-use. Refraining from activities and maintaining a non-fat diet were themes concerning lifestyle modifications. Some children spontaneously reported worries about the possibility of dying and frustration about being different from peers. Children coped with these worries by expressing faith in the effectiveness of medication, trying to be similar to peers or, in contrast, emphasizing their 'being different'. Children generally appeared effective in the way they coped with their carrier status and its implications. However, children who tested positive for

LQTS seemed most vulnerable. No significant differences in quality of life scores were found between carrier children and the reference group of Dutch children.

Nevertheless, dealing with the daily implications of their condition remains difficult in some situations, warranting continued availability of psychosocial support.

### S03.2

#### Uptake of genetic counselling and predictive DNA testing in hypertrophic cardiomyopathy

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**Introduction:** Hypertrophic cardiomyopathy is a common autosomal dominant disease, associated with heart failure and arrhythmias predisposing to sudden cardiac death. After the detection of the causal mutation in the proband predictive DNA testing of relatives is possible (cascade screening). Prevention of sudden cardiac death in patients with a high risk by means of an implantable cardioverter defibrillator is effective.

**Methods and results:** In 97 hypertrophic cardiomyopathy families with a sarcomere gene mutation we retrospectively determined uptake of genetic counselling and predictive DNA testing in relatives within one year after the detection of the causal mutation in the proband. Uptake of genetic counselling was 39% and did not differ significantly by proband's or relative's gender, nor by young age of the relative (<18 years) or a family history positive for sudden cardiac death. In second degree relatives, eligible for predictive DNA testing when the first degree relative had died, uptake was 27.5% ( $p=0.047$ ). Uptake of predictive DNA testing was 39%; conditional uptake of predictive DNA testing was 99%.

**Conclusions:** Uptake of genetic counselling in hypertrophic cardiomyopathy is comparable to uptake in oncogenetics. Conditional uptake of predictive DNA testing, however, is much higher. Because sudden cardiac death can be prevented uptake of genetic counselling in hypertrophic cardiomyopathy should be as high as possible. To achieve this research into the determinants of uptake is needed.

### S03.3

#### Genetic testing in familial cardiomyopathies: the cardiologists view

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Cardiomyopathies (CMs) are primary disorders of the myocardium. The most common subtypes, hypertrophic (HCM) and dilated (DCM) cardiomyopathy, are characterized by an abnormal thickening of the left ventricular wall with a preserved systolic function (HCM) and an enlarged left ventricular cavity with a reduced systolic function (DCM). HCM's and familial forms of DCM are monogenic heritable diseases. Mutations in 11 different genes encoding for contractile proteins have been identified and can be detected in 30 to 60% of the patients with HCM. Fortunately 3 genes (MYBPC-3, MYH-7 and TNNT-2) account for more than 60% of the known mutations.

In patients with familial DCM mutations are equally dispersed over 20 genes. This heterogeneity seriously hampers the feasibility of genetic screening. The only exception are patients with familial DCM with conduction system disease, in whom defects in the gene encoding for lamin A/C are found in 30 to 50%.

The clinical relevance of genetic testing in familial CMs is currently confined to 3 areas. 1) Lifestyle advice (sports, profession,...) in asymptomatic children and adolescents, 2) guiding prophylactic implantation of an implantable cardioverter defibrillator in patients with a family history of premature sudden death and 3) pre-implantation diagnosis in families with highly symptomatic forms of CM.

A promising area, almost unexplored today, is that of prophylactic "heart failure" therapy in asymptomatic carriers of DCM.

So far we have no human studies exploring how to influence the natural history of HCM, but it is clear that the relevance of early genetic diagnosis will explode once we understand how to modify disease progression.

As genetic testing can transform a healthy person into a patient, the role of dedicated clinical geneticists and psychologists in the treatment of these people cannot be overestimated.

### S03.4

#### Peer Support: A Critical Resource

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In the UK there has been a gap between what the National Health Service has provided and what individuals affected by young sudden cardiac death and inherited cardiovascular diseases require. Founded in 1995, the charity Cardiac Risk in the Young (CRY) has developed two unique services to address this disparity; the Bereavement Support Network and the Surgery Supporters Network. Both aim to be fast, expert and individual.

CRY's Bereavement Support Network is designed to facilitate "talking out" personal grief. It is based around a network of bereavement supporters who have each been affected by a young sudden cardiac death and subsequently completed a 2 year BAC accredited skills and theory course. Bereaved families can also attend an Annual Family Medical Conference in London and a series of Regional Bereavement Support Days around the UK. Furthermore, to facilitate the understanding of the potential genetic risks to other family members, CRY provides specialist cardiac information and funds expert cardiac pathology - ensuring uncertainty and time delays are minimised.

The CRY Surgery Supporters Network is designed for young people (aged 35 and under) who have had, or are about to confront, potentially life-saving surgery and want to talk to others who have been through a similar experience. These experiences include the fitting of pacemakers and implantable defibrillators, and ablation surgery. It was developed as a support system that increases effective coping and decreases social isolation for young implant recipients, whilst also offering support to others in a similar situation. Group meetings include; 'Question and Answer' sessions with a specialist cardiologist; group counselling; and opportunities to socialise.

It is important that the implementation of counselling for the specific psychological needs of those affected by inherited cardiovascular conditions are carefully considered. Counselling is only a luxury for those that do not need it.

### S04.1

#### Targeted treatment of tuberous sclerosis using mTOR inhibitors

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Inactivating mutations of *TSC1* or *TSC2* cause the inherited disorder tuberous sclerosis. They lead to loss of GAP activity of the TSC1/2 complex for the small GTPase Rheb and thereby to inappropriate activity of mTOR signalling that appears critical to the pathogenesis of many disease manifestations. mTOR inhibitors represent clear candidate therapies targeted to the molecular pathology of tuberous sclerosis and they have been used in a number of pre-clinical and early phase clinical trials. These have demonstrated reduction in size of TSC-associated renal tumours (angiomyolipomas) and improvement in lung function in patients with lung manifestations (lymphangioleiomyomatosis, LAM). A case-series of TSC-associated brain tumours (sub-ependymal giant cell astrocytomas) were also reported to shrink during rapamycin therapy. An important though variable feature of the tuberous sclerosis phenotype is learning difficulty. Recent studies in mouse models carrying heterozygous *TSC2* mutations demonstrated improvement in learning deficits following treatment with the mTOR inhibitor rapamycin. These promising pre-clinical and early-phase human trials have led to development of larger scale randomised control trials of mTOR inhibitors for the treatment of the renal, lung, and brain manifestations of TSC-associated disease.

### S04.2

#### Genetic aetiology defines optimal treatment in monogenic diabetes

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In the pre-genetics era there were two types of diabetes; type 1 which required insulin and type 2, usually treated with oral hypoglycaemic agents. Mutations in 8 genes are now known to cause isolated diabetes and there are at least 15 syndromes which include diabetes.

Monogenic diabetes accounts for 1-2% of diabetes with an estimated prevalence of up to 1/1000 in Europe. For some patients, defining the genetic aetiology of their diabetes may lead to improved treatment. Mild fasting hyperglycaemia resulting from heterozygous inactivating *GCK* mutations does not usually require pharmacological treatment and children misdiagnosed as having type 1 diabetes have been able to stop insulin injections without a deterioration in glycaemic control. Those patients with *HNF1A* or *HNF4A* mutations are sensitive to low dose oral sulphonylureas but may later progress to insulin. Heterozygous activating mutations in the *KCNJ11* and *ABCC8* genes encoding the Kir6.2 and SUR1 subunits of the beta-cell ATP sensitive potassium ( $K_{ATP}$ ) channel account for around 50% of patients with permanent neonatal diabetes (PNDM) diagnosed in the first 6 months of life. This discovery of the underlying genetic aetiology has dramatically improved treatment since most patients achieve improved glycaemic control upon transfer from insulin to high dose oral sulphonylureas. The challenge now is to educate health care professionals to recognise monogenic subtypes of diabetes in order to maximise the benefits of a pharmacogenetic approach to treating diabetes.

#### S04.3 MicroRNAs: Functions in metabolism and therapeutic opportunities

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#### S05.1 Structural genomic variation

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#### S05.2 Array-CGH in clinical practice: Fascination and frustration.

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Array-CGH gradually is being integrated into the routine clinical practice to detect submicroscopic chromosomal imbalances. The first experience is one of fascination. In about 15% of cases with a "chromosomal" phenotype, an etiological diagnosis can now be reached. Mosaicism can be detected more easily, and chromosomal aberrations are characterized precisely. Improved genotype-phenotype correlations not only facilitates counselling and guidance, but also has lead to the elucidation of the genetic cause of several clinical disorder such as CHARGE and Pitt-Hopkins syndromes. More surprisingly, the field of reverse phenotyping has emerged, where phenotyping of individuals sharing a similar imbalance has resulted in the delineation of novel recognisable entities.

Now that the major technical issues of array-CGH have been solved, its application in clinical practice faces several challenges. In an attempt to decipher the large number of remaining unexplained cases, higher resolution arrays were applied, but met with limited success. It appears that many smaller imbalances cause a monogenic disorder, which had not been recognized clinically. The high number of rare variants poses major difficulties in the interpretation of found imbalances. Traditional cytogenetic criteria of pathogenicity such as size or *de novo* occurrence do not longer apply. Also, the distinction between pathogenic and polymorphic becomes blurred even further since many CNV's act as susceptibility loci and in addition, the associated phenotypes vary in an unexplained way. Also, as a result of genome wide screening, the clinician will be confronted with unexpected results that may have important implications for the person and his family. These issues will have to be considered before one can apply array-CGH in prenatal diagnosis.

#### S05.3 Mitotic recombination in leukaemia

**B. D. Young, M. Raghavan, M. Gupta:**

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The introduction of array-based analysis of single nucleotide polymorphisms (SNPs) allows the rapid determination of many thousands of allelotypes in a human DNA sample. This technology is particularly suited to the study of changes that take place during the development of cancer. For example, regions of tumour-associated loss of heterozygosity (LOH) can be detected as by comparison with germline DNA from the same individual. Since this approach also yields copy number information it is possible to determine the probable source of LOH. The application of SNP genotype arrays to acute myeloid leukaemia (AML) unexpectedly uncovered large regions of acquired homozygosity which were not associated with copy number gain or loss and which cannot be detected by conventional cytogenetics. This phenomenon, which often continues to the telomere, has been termed acquired uniparental disomy (aUPD) and is the consequence of a mitotic recombination event occurring during the evolution of the cancer. In a large study we have demonstrated that there is a distinct pattern of aUPD in approximately 17% of AML with certain regions being preferentially affected. Furthermore we have shown that certain gene mutations are being rendered homozygous by the mitotic recombination event, including *FLT3*, *WT1* and *CEBPA*. These data strongly suggest that the mutation precedes mitotic recombination [1].

A relatively large proportion of AML patients will relapse with resistant disease. Recently we have applied SNP array analysis to serial leukaemia samples including both presentation and relapse material in order to determine whether mitotic recombination could have a role in disease evolution. It was observed that up to 40% of AML at relapse should evidence of aUPD frequently affecting chromosome 13q and involving mutations to *FLT3*. This suggests that mitotic recombination events could have an important role in the evolution of AML [2]

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#### S06.1 Genetics of human autoimmune disease

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#### S06.2 Genome-wide association studies of obesity and height: What have we learned?

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Genome-wide association (GWA) studies have been used to map loci at which common genetic variants are associated with polygenic traits and diseases. However, the modest effects of these common variants has meant that mapping and validating associated loci requires large numbers of samples and collaborations. We have taken a collaborative approach and are using GWA studies representing over 100,000 individuals to identify common variants associated with anthropometric traits, including height and measures of obesity. Earlier iterations of these efforts, involving studies of up to 30,000 samples, successfully identified 10 loci for body mass index and over 40 loci for height. These results have implicated both expected and novel biological pathways, and have highlighted the role of central nervous system in regulating body weight as well as several distinct pathways in the regulation of

height. These studies have moved us closer to our primary goal, which is to shed light on the underlying biology of the regulation of height and weight in human populations.

Thus far, the associated variants we have identified explain only a small fraction of the expected contribution of genetic factors to population variation. Clearly, many of the causal loci and even causal variants at known loci remain to be discovered. Thus, further efforts and analyses will likely uncover additional genetic determinants, although the relative contributions to trait variation of common and rare variants, and discovered vs undiscovered loci, remains to be seen. These efforts will include expansion of GWA studies both in sample size and in the number and types of variants that can be assayed, studies of individuals samples from the extremes of trait distributions, comprehensive and deep sequencing of known loci, more complex analyses, and, eventually, comprehensive sequencing of the genome in large numbers of individuals.

### S06.3

#### Low-penetrance genes for colorectal cancer predisposition

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Recent large-scale screens have shown that the common disease-common variant genetic model is correct for the major cancers. Several SNPs have now been associated with a differential risk of breast, colorectal and prostate carcinomas, and evidence for similar predisposition to other tumour types is accumulating. There appear to be several different mechanisms of raising cancer risk, but some prime candidate genes, such as those involved in DNA repair, are strikingly absent to date. The relative risks associated with cancer susceptibility SNPs are modest (typically up to 1.3-fold) and the variants detected to date can account for a small proportion of the familial clustering of cancer. The remaining risk may be explained by other types of genetic variant, including copy number polymorphisms and rare (or "private") alleles with modest effects on disease risk.

### S07.1

#### Silencing of the FMR1 gene in human embryonic stem cells generated from fragile X blastocysts

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### S07.2

#### Trinucleotide instability and DNA repair

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### S07.3

#### Myotonic dystrophy: Complex repeats in a complex disorder

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Myotonic dystrophy is an extremely variable disorder. Ages of onset vary from birth to old age and the symptoms range from mild to se-

verely debilitating and life threatening. Although primarily neuromuscular, the precise array of symptoms observed in any one patient varies dramatically. Myotonic dystrophy type 1 is caused by the expansion of an unstable CTG repeat with patients typically inheriting from 50 to several thousand copies of the repeat. In general, longer alleles are associated with more severe symptoms and an earlier age of onset. However, explanations for the additional symptomatic variability not accounted for by repeat length have remained enigmatic. In order to try and understand symptomatic variation in myotonic dystrophy we have been analysing an unusual family in which the typical myotonic dystrophy symptoms are accompanied by an intermediate Charcot-Marie-Tooth peripheral neuropathy, acute encephalopathic attacks and deafness. We have determined that in addition to the typical CTG repeat expansion, this family has a complex repeat expansion at the 3' end of the array comprised of CGG, CCG and CTG repeats. These variant repeats presumably mediate the additional symptoms by an RNA gain of function analogous to that observed in fragile-X associated tremor ataxia syndrome. We have also determined that similar such variant repeats are observed in other myotonic dystrophy families and appear to be associated with decreased genetic instability and less severe symptoms. Notably, variant repeats are clustered at the 3' end of the array and have not been detected at the 5' end of the array. More bizarrely, variant repeats in some families appear to have arisen *de novo*. The occurrence of such *de novo* base substitutions reveals a mutation frequency several orders of magnitude greater than previously observed at any human loci and further extends our knowledge of the unusual sequence properties of expanded repeats.

### S08.1

#### RET as a diagnostic and therapeutic target in MEN2

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The RET gene encodes a receptor tyrosine kinase that is expressed in neural crest-derived cell lineages. The RET receptor plays a crucial role in regulating cell proliferation, migration, differentiation, and survival through embryogenesis.

Activating mutations in RET lead to the development of several inherited and noninherited diseases. Germline point mutations are found in the cancer syndromes multiple endocrine neoplasia (MEN) type 2, including MEN 2A and 2B, and familial medullary thyroid carcinoma. These syndromes are autosomal dominantly inherited. The identification of mutations associated with these syndromes has led to genetic testing to identify patients at risk for MEN 2 and familial medullary thyroid carcinoma and subsequent implementation of prophylactic thyroidectomy in mutation carriers.

The RET mutations induce oncogenic activation of the RET tyrosine kinase domain via different mechanisms, making RET an excellent candidate for the design of molecular targeted therapy. Recently, various kinds of therapeutic approaches, such as tyrosine kinase inhibition have been developed and tested.

### S08.2

#### Li Fraumeni syndrome: The role of DNA copy number variation in cancer susceptibility

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DNA copy number variations (CNVs) are a significant form of inter-individual genetic variation. The size and plasticity of CNV regions makes them particularly intriguing to the study of cancer. Li-Fraumeni syndrome (LFS) is an autosomal dominant disorder, frequently associated with germline mutations of the TP53 tumor suppressor gene. LFS is clinically and genetically heterogeneous and TP53 mutational status alone does not explain the cancer phenotype in these families. The genetic events that determine specific cancers in each family are unknown. Further, many LFS families do not have detectable TP53 missense mutations. We conducted a genome-wide profile of CNVs in DNA of peripheral blood lymphocytes of LFS families (Shlien A, et al 2008). Using the Affymetrix 500K array, we previously examined genomic DNA from a large healthy population (n=770) and an LFS cohort (n=53) and showed that the number of CNVs per genome is largely

invariable in the healthy populations, but increased in these cancer-prone individuals ( $p=0.01$ ). Expanding on the initial analysis and using multiple algorithms and a replication cohort of LFS DNA ( $n=22$ ) hybridized on Affymetrix 6.0GW high resolution arrays we now demonstrate that: 1) CNV deletions are more frequent than duplications in mutation carriers ( $p=3.28 \times 10^{-5}$ ); 2) the overall number of CNVs exceeds 300 in carriers (154 in controls); and 3) the difference in CNVs persists in these individuals' tumors. We have also recently uncovered rare CNV deletions at 17p13.1 in LFS patients ( $n=4$ ) and in patients with multiple congenital abnormalities but no cancer phenotype ( $n=4$ ). This led us to posit that structural features of specific CNVs in the region may play a role in distinguishing a cancer from a non-cancer phenotype. The CNVs at 17p13.1 all include *TP53* but range in size from 4.236 to 2Mbp. The large deletions, causing haploinsufficiency of nearly 40 genes, are associated with a broad phenotype that includes multiple congenital abnormalities, while smaller focal *TP53* deletions (from exons 2 to 11) are associated with cancer susceptibility. Using qPCR and long-range PCR, we continue to fine map these deletions with the goal of finding the sequence features and mechanisms which cause these large genomic events (e.g. LCR-mediated NAHR). Our results suggest an important role of DNA structural variation in cancer susceptibility, and these models will be discussed during this presentation.

### S08.3

#### Familial gastric cancer

C. Caldas;

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### S09.1

#### Molecular karyotyping: From postnatal to preimplantation genetic diagnosis?

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Molecular karyotyping or genome wide array CGH has been implemented in postnatal diagnosis of patients with idiopathic mental retardation and congenital anomalies and is challenging conventional karyotyping as the prime diagnostic tool. Despite its successes, interpretation of the results coming from arrays with ever increasing resolution is becoming the main challenge. I will demonstrate how "Mendelian copy number variants" - apparently benign CNVs that can cause a disease phenotype dependent on copy number state, sex and genetic or environmental background - require large scale collaborative efforts to collect sufficient data and the development of expert systems to provide accurate diagnosis. The technology has, more recently, been applied in a prenatal diagnostic setting. I will illustrate how the technology helps prenatal diagnosis, but also demonstrate the potential risks of using this technology. Finally, we developed a novel tool to genome wide screen CNV and SNP-genotype single cells. When applied to cleavage stage embryos from young fertile couples we discovered, unexpectedly, an extremely high incidence of chromosomal instability, a hallmark of tumorigenesis. Not only mosaicism for whole chromosome aneuploidies and uniparental disomies but also frequent segmental deletions, duplications and amplifications that were reciprocal in sister blastomeres were detected in most cleavage stage embryos implying the occurrence of breakage-fusion-bridge cycles. As a consequence, PGD-AS will not improve the selection of genetically normal embryos. This not only explains the low human fecundity but also identifies postzygotic chromosomal instability as a leading cause of constitutional chromosomal disorders.

### S09.2

#### Prenatal diagnosis and fetal treatment using fetal RNA in maternal body fluids

D. Bianchi;

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Cell-free fetal (cff) DNA in maternal plasma provides a noninvasive source of fetal genetic material. Cff DNA is elevated in preeclampsia, placental abnormalities, and fetal aneuploidy. Qualitative analysis of cff DNA in maternal plasma is already in clinical use worldwide for noninvasive prenatal diagnosis of Rhesus D and fetal gender. Excit-

ing work has recently been published that suggests that cff DNA and RNA in maternal plasma can facilitate noninvasive prenatal diagnosis of trisomies 18 and 21, using ratios of single nucleotide polymorphisms (SNPs) or using a shotgun sequencing approach. In our laboratory we are performing comparison gene expression microarray analyses between the pregnant woman and her newborn to detect fetal gene sequences that are indicative of normal and abnormal fetal development in the second and third trimesters (Maron et al. *J Clin Invest* 2007; 117:3007-3019). Amniotic fluid supernatant is also a rich source of cell-free fetal DNA and RNA; it can provide novel information about gene expression and functional development in the living human fetus. We have generated preliminary data on fetal gene expression from cell free mRNA in amniotic fluid. This has led to the identification of novel differentially-regulated genes, and disrupted biologic pathways in various fetal pathologies such as twin to twin transfusion syndrome, fetal hydrops, and trisomy 21. Functional genomic analysis of second trimester fetuses with trisomy 21 suggests that oxidative stress, ion transport, and G-protein signaling are important. Most recently, we have used the Connectivity Map to generate testable hypotheses regarding fetal treatment with small molecule drugs. The Connectivity Map "connects" disease states, biological systems disruption (as measured by pathway analysis), and pharmaceutical compounds to treat the disease. This allows a true translation from bench to bedside, and suggests a possible continuum between prenatal diagnosis and fetal therapy. Supported by National Institutes of Health R01 HD42053-06.

### S09.3

#### The challenge of prenatal and preimplantation genetic diagnosis of mitochondrial disorders

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Mitochondrial diseases are caused by defects in the oxidative phosphorylation. They can be caused by mutations in nuclear or mitochondrial DNA (mtDNA) encoded genes. Mutation analysis of nuclear genes in prenatal (PND) or preimplantation genetic diagnosis (PGD) is routine. Pitfalls do occur in biochemical analysis in PND. Mutations in the mtDNA lead to a wide spectrum of diseases with very variable clinical expression. They are transmitted exclusively maternally and are usually heteroplasmic. Severity of symptoms is partially determined by mutation load. PND or PGD for mtDNA mutations is complex and experience is limited. Prerequisites for reliable PND of mtDNA mutations have been formulated by Poulton and Turnbull (2000) and include: 1. a close correlation between mutation load and disease manifestation 2. no significant time-dependent changes in mutation load 3. a uniform distribution of mutation load in different tissues. These criteria also apply for PGD.

For genetic counseling one may subdivide the mitochondrial mutations in 5 categories: 1 *de novo* mutations. Recurrence risk is low and PND or PGD can be offered for reassurance. 2. Inherited stable mutations, such as the m.8993T>G/C mutations (leading to NARP/Leigh syndrome). Outcome is favourable for this mutation when the mutation load is < 60%, while mutation load >90% is associated with a bad prognosis. Prediction of severity in the grey zone (60-90%) is difficult, but a tendency for percentages at the extremes has been observed in oocytes, which would favour conclusive results. PND was offered more than 10 times. PGD for the m.8993T>G mutation has been reported once. 3. Inherited unstable mutations. The classical example is the m.3243A>G (MELAS) mutation. There is a certain correlation between mutation load and clinical severity but individual exceptions exist. It is impossible to define a completely safe lower threshold. A limited number of PNDs have been carried out. Mutant load was found to be fairly stable in CVS and amniotic cells. Oocytes and foetuses of carrier women can be without mutant load, the number dependent on the mutation load of the carrier. PGD for the 3243A>G mutation was carried out by our group (unpublished results). Mutation load showed a broad range between embryos, but was equal in the blastocysts of one embryo. Some embryos had a fairly low mutation load. 4. Rare mutations with unknown outcome. Insufficient information is available for reliable predictions. 5. Homoplasmic mutations. PND or PGD is useless as 100% of the mtDNA is mutated.

In conclusion, assessment of mtDNA mutation load in chorionic villi, amniotic cells or blastocysts is quite accurate nowadays and prelimi-

nary data show that the mutation percentage is stable at different time points in pregnancy or in different cells of one embryo. However, PND for mitochondrial mutations is complicated by interpretation of test results, particularly in case of intermediate results. PGD is probably a more acceptable option to exclude or reduce the risk of a severely affected child, but experience is still very limited.

### S10.1

#### Alzheimer disease

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### S10.2

#### Pathways to Parkinsonism

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Parkinson disease (PD) was regarded as the archetypal non-genetic disease for many years but discoveries over the past decade have shown that this is not true. There are two genes that cause autosomal dominant PD, LRRK2 (leucine-rich repeat kinase 2) and SNCA. The latter gene is interesting because the protein product,  $\alpha$ -synuclein, is deposited in Lewy bodies, the characteristic pathological inclusion of PD and a related disease, dementia with Lewy bodies. LRRK2 cases also usually have Lewy bodies, suggesting that these two genes might be related to one another. The mechanistic basis of this relationship is currently unclear, but both LRRK2 and  $\alpha$ -synuclein are reported to have roles in neuronal function. LRRK2 has attracted a great deal of interest as a possible drug target as it has been shown that the kinase activity is required for pathogenic gain of function effects, at least in cell culture models. LRRK2 is also a relatively common cause of inherited disease in several populations, although the decreased penetrance of many mutations has probably contributed to an underestimation of the genetic contribution to PD in prior studies.

Three genes have been reported to cause autosomal recessive parkinsonism, a milder phenotype with earlier onset than typical PD where Lewy bodies are not always present. Recent data suggests that the oxidative stress sensor DJ-1, the mitochondrial kinase PINK1 and the E3 protein ubiquitin ligase parkin all play roles in cellular responses to oxidative stress and possibly by influencing mitochondrial function. This indicates that recessive parkinsonism genes are probably found in a common pathway, although whether this is related to the dominant PD pathway is uncertain. There are a number of additional genes (PANK2, PLA2G6, ATP13A2, FBXO7, TAF1, and PRKRA) that cause parkinsonism/dystonia with or without Lewy bodies, whose relationship to 'PD' is also unclear.

As well as mendelian genes, several robust associations between PD and common genetic variants have been reported. These are currently being supplemented by data from genome-wide association studies. Although the effect size of most common polymorphisms are quite modest, with odds ratios in the range of 1.2-1.4, they suggest that even sporadic PD has a partial genetic basis. In this talk, the identity of the most robust associations will be discussed as they indicate that the genetic determinants for PD are more likely related to dominant PD genes, with some interesting additions that are more often thought of as being associated with other neurodegenerative disorders. Overall, this data is painting a complex picture of genetic contributions to an apparently simple non-genetic disease that leads to a rethinking of views about etiology and pathology in PD.

### S10.3

#### Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by progressive wasting and weakness of limb, bulbar, and respiratory muscles. The disease is caused by loss of motor neurons in the spinal cord, brainstem, and motor cortex and can occur at any time in adulthood, with a median age of onset in the mid fifties. The incidence (around 1-2 per 100,000 person-years) and prevalence (4-

13 per 100,000) of ALS are remarkably similar across European and North American populations. About half of patients die within 3 years of symptom onset, usually because of respiratory failure. The only therapeutic strategy to slow the disease is currently riluzole, which delays progression by 3-6 months.

In epidemiological studies 1-13% of ALS patients are claimed to have a familial disposition for the syndrome (FALS), most commonly with a Mendelian dominant mode of inheritance. Linkage analysis in 18 familial ALS pedigrees associated the gene encoding Cu/Zn superoxide dismutase (*SOD1*) on chromosome 21 to the syndrome. Mutational analysis revealed point-mutations in the *SOD1* gene to co-segregate with the disease in these pedigrees. Subsequent studies have identified over 140 different *SOD1* mutations in ALS patients. Mutations in *SOD1* can be identified in approximately 12 - 23 % of the patients diagnosed with FALS and in 1 - 4 % of patients diagnosed with SALS patients. Other genes linked to 1-3% of familial ALS are ANG, ALS2, VAPB, TARDBP and FUS.

In the remaining 90 % of ALS patients where there is no family history and the disease is considered to be sporadic (denoted as SALS). Although the genetic contribution to sporadic ALS appears to be considerable with an estimated heritability ranging 0.35-0.85, the genetics of this disease remain largely unknown. Candidate gene studies have identified potential risk factors, but replication of these associations has proven to be difficult. Several genome-wide association (GWA) studies have now been performed in sporadic ALS and have highlighted three novel candidate genes (*ITPR2*, *FGGY* and *DPP6*).<sup>3-9</sup> Two independent studies found nominal p-values < 0.05 for *DPP6*,<sup>6,10</sup> but recent genome-wide efforts could not confirm this association.<sup>7,9</sup> The GWA studies in ALS have been relatively small and as a result of limited power may have generated potentially spurious results. In order to identify truly associated genetic risk factors for ALS, we recently performed a two-stage GWA study encompassing 19,838 subjects. An initial genome-wide screen with 2,323 cases and 9,013 controls was followed by a replication experiment of all SNPs with a p-value < 1.0x10<sup>-4</sup> in an independent cohort of 2,532 cases and 5,940 controls. Genotyping experiments were performed using Illumina BeadChips and KASPar genotyping technology. Two new candidate loci were identified and will be presented.

### S11.1

#### Novel insights and challenges from the inborn errors of vitamin B12 metabolism

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Over the last few years the discovery of the *MMACHC*, *MMADHC*, and *LMBRD1* genes responsible for the *cbIC*, *cbID*, and *cbIF* inborn errors of vitamin B<sub>12</sub> metabolism has shed more light on the early steps of intracellular handling of this vitamin. The *LMBRD1* gene product is responsible for the transport of cobalamin out of the lysosome. The *MMACHC* gene product binds cobalamin, and has been suggested to play a role in reduction of cob(III)alamin to cob(II)alamin, removal of the upper axial ligand and production of the base-off conformation. Epigenetic modification of the *MMACHC* promoter is associated with a malignant phenotype in a human melanoma cell line. *Mmachc* knockout mice do not survive to term and embryos show neurological abnormalities consistent with the proposed requirement for vitamin B<sub>12</sub> to prevent neural tube defects. The *MMADHC* gene product appears to play a role in directing cobalamin to the mitochondria or the cytoplasm. Mutations affecting different domains of the *MMADHC* gene product may result in decreased synthesis of AdoCbl alone, of MeCbl alone, or of both cobalamin coenzyme derivatives.

The recent expansion of newborn screening has resulted in a number of surprising findings. These include mothers newly diagnosed with an inborn error of cobalamin metabolism on the basis of a positive screening test in their newborn, infants with cobalamin deficiency on the basis of subclinical cobalamin deficiency in their breast-feeding mothers, infants with a positive newborn screening test who carry mutations previously shown to cause adult onset disease, and a newborn with the first mutation in the gene for the transcobalamin receptor.

**S11.2****Clinical, biochemical and genetic aspects of the methylmalonic acidemias****M. R. Baumgartner;***Division of Metabolism, University Children's Hospital, Zürich, Switzerland.*

Methylmalonic acidurias (MMAurias) are a heterogeneous group of inborn errors of metabolism biochemically characterized by the accumulation of methylmalonic acid (MMA) in body fluids and tissues. They result from deficiency of methylmalonyl-CoA mutase apoenzyme (MCM) or by a defect in the synthesis of its cofactor adenosylcobalamin (AdoCbl). MCM catalyzes the conversion of L-methylmalonyl-CoA to succinyl-CoA thus linking the final catabolic pathways of L-isoleucine, L-valine, L-methionine, L-threonine, odd-chain fatty acids, and cholesterol side chains to the tricarboxylic acid cycle.

Several mutant genetic classes that cause *isolated* MMAuria are known, based on biochemical, enzymatic and genetic complementation analysis. The deficiencies of the apomutase locus are further subdivided into defects without ( $mut^0$ ) and with residual activity ( $mut^+$ ). The *cblA*, *cblB* and the variant 2 form of *cblD* complementation groups are linked to processes unique to AdoCbl synthesis. The *cblC*, *cblD* and *cblF* complementation groups are associated with defective methyl-cobalamin synthesis as well resulting in *combined* MMAuria and homocystinuria. Mutations in the genes associated with most of these defects have been described. Finally, a few patients have been described with mild MMAuria associated with mutations of the methylmalonyl-CoA epimerase gene or with neurological symptoms due to *SUCL* mutations.

The clinical presentation of affected patients is variable. The majority of patients present during the newborn period or infancy with life-threatening metabolic crises resulting in multi-organ failure or even death. These crises are often precipitated by catabolic stress, e.g. induced by febrile illness. Severe keto- and lactic acidosis, hypo- or hyperglycemia, neutropenia, hyperglycinemia, and hyperammonemia are the most common laboratory findings. In a subgroup of patients chronic progressive disease, psychomotor retardation, and failure to thrive are the leading symptoms. Although the overall survival has improved during the last two decades, long-term outcome still remains disappointing. Neurological outcome is often impaired by extrapyramidal movement disorder and developmental delay. Furthermore, chronic renal failure is frequently found. Patients with  $mut^0$  and *cblB* have an earlier onset of symptoms, a higher frequency of complications and deaths, and a more pronounced urinary excretion of methylmalonic acid than those with  $mut^+$  and *cblA*-defects. Reliable classification of these patients is essential for ongoing and future prospective studies on treatment and outcome.

**S11.3****Homocysteine/folate and neural tube defects****H. J. Blom;***VU University Medical Center, Department of Clinical Chemistry, Metabolic Unit, Amsterdam, The Netherlands.*

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**S12.1****Using naturally-occurring mutations to identify stem cell niches, trace cell lineage and the origins of cancer in humans****N. Wright;***Histopathology Lab, London Research Institute, Cancer Research UK, London, United Kingdom.*

There have been considerable advances in our understanding of the organisation of stem cells in epithelial tissues. The identification of reliable stem cell markers has allowed lineage tracing in some tissues, such as the intestine. But these observations are currently confined to experimental animals, and the genetic manipulation required is not available in humans. We have used a series of naturally-occurring genetic alterations in humans to infer what we think are interesting observations about human stem cells and the origins of human cancer. It is widely accepted that tumors are monoclonal in origin, arising from a mutation or series of mutations in a single cell and its descendants. The clonal origin of colonic adenomas and uninvolved intestinal mucosa from an XO/XY mosaic individual with familial adenomatous polyposis (FAP) was examined directly by *in situ* hybridization with Y

chromosome probes. In this patient, the crypts of the small and large intestine were clonal, showing that each was derived at some stage from a single cells. However, at least 76 percent of the microadenomas were *polyclonal* in origin (*Science* 272:1187-90). Previously studies using X-linked genes such as glucose-6-phosphate dehydrogenase have been handicapped by the need to destroy the tissues to study the haplotypes of glucose-6-phosphate dehydrogenase, but we were able to directly visualize X-inactivation patches in human females heterozygous for the G6PD Mediterranean mutation (563 C-T). The results showed again that crypts were clonal but crypts formed large families (*Proc Natl Acad Sci USA* 100:3311-3314).

We now describe a technique for detecting the expansion of a single cell's progeny that contain clonal mitochondrial DNA (mtDNA) mutations affecting the expression of mtDNA-encoded cytochrome c oxidase (COX). Since such mutations take up to 40 years to become phenotypically apparent, we believe these clonal patches take origin in stem cells. Dual-color enzyme histochemistry was used to identify COX-deficient cells and mutations confirmed by microdissection of single cells with polymerase chain reaction sequencing of the entire mtDNA genome. These techniques have been applied to human intestine, liver, pancreas and skin. Our results suggest that the stem cell niche is located at the base of colonic crypts and above the Paneth cell region in the small intestine, in accord with dynamic cell kinetic studies in animals. In the pancreas, exocrine tissue progenitors appeared to be located in or close to interlobular ducts, and in the liver, we propose that stem cells are located in the periportal region. In the skin, the origin of a basal cell carcinoma appeared to be from the outer root sheath of the hair follicle. We propose that this is a general method for detecting clonal cell populations from which the location of the niche can be inferred, also affording the generation of cell fate maps, all in human tissues. The technique also allows analysis of the origin of human tumors from specific tissue sites (*Proc Natl Acad Sci USA*; 103:714-9; *Stem Cells* (in press)).

**S12.2****Mapping mRNA expression QTLs in hematopoietic stem cells and their progeny****G. de Haan;***Department of Cell Biology, Section Stem Cell Biology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.*

A fundamental problem in biology is how a single genome can lead to widely different cellular phenotypes. An illustrative and clinically relevant example is the generation of all mature blood cells types from a small population of hematopoietic stem cells. Identification of gene networks specifying stemness or lineage commitment is of major relevance for the emerging fields of tissue engineering and regenerative medicine. We developed a genetical genomics approach as a tool to dissect networks of interacting genes that specify cellular function in four developmentally distinct hematopoietic cell stages. We evaluated genome-wide RNA transcript expression in highly purified Lin<sup>-</sup>Sca-1<sup>+</sup>ckit multilineage cells, committed Lin<sup>-</sup>Sca-1<sup>+</sup>ckit progenitor cells, erythroid Ter119<sup>+</sup> and myeloid Gr1<sup>+</sup> precursor cells isolated from a large pedigree of genetically related and fully genotyped BXD recombinant mouse strains. Variation in transcript abundance across all strains and in all cell types was assessed by Illumina Sentrix Mouse-6 chip technology, interrogating ~47,000 probesets mapping across the genome. For each variably expressed transcript genetic linkage analysis identified a quantitative trait locus that affects variation in expression levels of the corresponding gene (eQTL). These eQTLs map in the vicinity of their target gene (*cis*-regulation), or map elsewhere in the genome (*trans*-regulation).

Complex transcript profiles for each cell type could be dissected into more simple individual gene networks, each consisting of transcripts whose variation in expression levels are regulated in trans by a single genomic locus. We identified 1316 regulatory loci with cell-stage-specific activity, including 140 loci that controlled gene expression predominantly in the most primitive hematopoietic cells. We performed hierarchical clustering of all genes that were consistently downregulated or upregulated during erythroid or myeloid development and detected multiple modules of co-varying transcripts for each set of consistently downregulated or upregulated transcripts. To reveal whether such modules of transcripts were under common genetic control, we performed eQTL clustering of 52 transcripts that were consistently down-

regulated during both erythroid and myeloid differentiation. A genetic analysis revealed co-localization of eQTLs for multiple transcripts, which confirms shared regulatory relationships. Interestingly, a substantial number of the 52 transcripts were regulated by loci on chromosomes 1, 5, 9, 12, and 18, consistent with earlier QTL studies which documented that these chromosomes contain loci that control the pool size or the turnover of primitive hematopoietic cells. In addition to gene expression data, we have recently embarked on studies aimed to determine whether micro-RNA abundance and DNA methylation patterns are regulated in a strain-dependent way during myeloid and erythroid development.

Our data illustrate how hematopoietic cell differentiation may be the result of cell-type dependent rewiring of transcriptional connections. This rewiring is observed as switches in correlation patterns, and indicates reversal of regulatory polarity due to cell-type-specific transcriptional co-factors. Our dataset leads to an unprecedented number of concrete predictions about the dynamic rewiring of the developmental regulatory network, which can be followed-up by hypothesis-driven experimentation.

#### S12.3

#### Stem cells as common ancestors in a colorectal cancer ancestral tree

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Stem cells can be defined in many ways. One retrospective way to define and characterize stem cells is by ancestry. A somatic cell ancestral tree has three types of cells. A start (zygote or first transformed cell), present day cells, and no longer present cells. No longer present cells can be divided into ancestors (with present day progeny) and dead-ends (no present day progeny). Functionally, ancestors are stem cells and dead-ends are non-stem cells. Therefore, a somatic cell ancestral tree is essentially a stem cell tree. In theory, molecular phylogeny can reconstruct ancestral trees by comparing variations between present day genomes. Genomes are almost perfect copies of prior copies, and the numbers of random replication error can be used to infer the time back to a common ancestor. Preliminary studies suggest that methylation patterns in certain CpG rich regions effectively function as epigenetic somatic cell molecular clocks. Methylation patterns sampled from normal colon crypts are consistent with a niche with multiple mitotic stem cells per crypt. Methylation patterns sampled from colorectal cancer glands are diverse and inconsistent with very rare cancer stem cells (CSCs). Simulations suggest that each cancer gland, similar to a normal crypt, is also maintained by a stem cell hierarchy and contain multiple CSCs. Potentially it may become practical to read the histories of human cells from their replication errors, which primarily accumulate in stem cells.

#### S12.4

#### Using microenvironmental microarrays to decipher the role of the niche signaling in breast progenitor cell fate

**M.A. LaBarge<sup>1</sup>, R. Villadsen<sup>2</sup>, O. Petersen<sup>2</sup>, M. J. Bissell<sup>1</sup>;**

<sup>1</sup>*Life Sciences Division, Lawrence Berkeley National Laboratory, University of California, Berkeley, CA, United States.* <sup>2</sup>*Department of Cellular and Molecular Medicine, Faculty of Health Sciences, and Zoophysiological Laboratory, Department of Molecular Biology, University of Copenhagen, Copenhagen, Denmark.* Previously we had shown that within the luminal population of the human breast, a subpopulation of cells resembling stem cells could be identified (1). These cells contained both luminal and myoepithelial characteristics. Since cellular pathways that contribute to adult human mammary gland architecture and lineages had not been described previously, we identified a candidate stem cell niche in ducts and zones containing progenitor cells in lobules (2). Putative stem cells residing in ducts were essentially quiescent, whereas the progenitor cells in the lobules were more likely to be actively dividing. Cells from ducts and lobules collected under the microscope were functionally characterized by colony formation on tissue culture plastic, mammosphere formation in suspension culture, and morphogenesis in laminin-rich extracellular matrix gels (LrECM). Staining for the lineage markers keratins K14 and K19 further revealed multipotent cells in the stem cell zone and three lineage-restricted cell types outside this zone. Multiparameter cell sorting and functional characterization with reference to anatomi-

cal sites *in situ* confirmed this pattern. The proposal that the four cell types are indeed constituents of an as of yet undescribed stem cell hierarchy was assessed in long-term cultures in which senescence was bypassed. These findings identified an adult human breast ductal stem cell activity and its earliest descendants.

Multi-potent progenitor cells are some of the most primitive members of the developmental hierarchies that maintain homeostasis. That progenitors and their more mature progeny share identical genomes suggests that fate decisions are directed by interactions with extrinsic soluble factors, ECM, and other cells, as well as physical properties of the ECM. To understand regulation of fate decisions, therefore, would require a means of understanding carefully choreographed combinatorial interactions. We used microenvironment protein microarrays to functionally identify combinations of cell-extrinsic mammary gland proteins and ECM molecules that imposed specific cell fates on bipotent human mammary progenitor cells. Micropatterned cell culture surfaces were fabricated to distinguish between the instructive effects of cell-cell versus cell-ECM interactions, as well as constellations of signaling molecules; and these were used in conjunction with physiologically relevant 3 dimensional human breast cultures. Both immortalized and primary human breast progenitors were analyzed (3). We report on the functional ability of those proteins of the mammary gland that maintain quiescence, maintain the progenitor state, and guide progenitor differentiation towards myoepithelial and luminal lineages. (4,5,6,7)

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#### S13.1

#### Reverse Phenotyping: Towards an integrated (epi)genomic approach to complex phenotypes and common disease

**S. Beck;**

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What determines a phenotype remains one of the most fundamental questions in biology and medicine. In addition to genetic factors, non-genetic factors such as epigenetic and environmental factors have been shown to play important roles. Of the epigenetic factors, methylation at CpG dinucleotides is the only known biologically relevant epigenetic modification at the DNA level in humans and vertebrates in general. Knowledge of the methylation status of each of the ~28 million CpG sites (methylome) in the haploid human genome is therefore of great importance for cellular identity, differentiation, development and, if perturbed, for disease aetiology. To understand the rules governing DNA methylation and the consequences if DNA methylation is perturbed requires genome-wide analysis of its temporal and spatial plasticity. Almost 60 years after the discovery of 5-methyl cytosine and about 25 years since the discovery that altered DNA methylation plays a role in disease (particularly in cancer), technologies for methylome analysis have finally become available.

I will present data from our efforts using sequencing- and array-based platforms for high-throughput DNA methylation analysis, discuss some of the lessons learnt and give an outlook on how the data may be used in an integrated approach - termed 'reverse phenotyping' - to analyse and better understand the (epi)genomics of phenotypic plasticity in health and disease.

For more details, please visit

<http://www.ucl.ac.uk/cancer/research-groups/medical-genomics>

## S13.2

**A novel genetic mechanism for Lynch syndrome resulting in heritable somatic methylation of MSH2**

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Lynch syndrome (HNPCC) patients are susceptible to colorectal, endometrial and a range of other cancers due to heterozygous inactivating mutations in one of the mismatch repair genes, *MLH1*, *PMS2*, *MSH2* or *MSH6*. In multiple patients with an *MSH2*-deficient tumor, in whom no germline mutation in *MSH2* could be detected, a heterozygous germline deletion of 4.9 kb encompassing the last exons of *EPCAM* (formerly known as *TACSTD1*) was found. *EPCAM* is located directly upstream of *MSH2* and encodes the epithelial cell adhesion molecule Ep-CAM. Due to the deletion of the transcription termination signal transcription of *EPCAM* was shown to extend into *MSH2*. As antisense transcription of CpG islands may lead to methylation, we tested whether the transcription of the *MSH2* promoter would lead to methylation of its CpG dinucleotides. Indeed, the *MSH2* promoter in *cis* with the deletion is methylated in Ep-CAM positive, but not in Ep-CAM negative, normal tissues, thus revealing a correlation between transcriptional read-through of the mutated *EPCAM* allele and epigenetic inactivation of the corresponding *MSH2* allele. A distinct deletion that also includes the 3' end of *EPCAM* was detected in two Chinese families, one of which was previously described with mosaic *MSH2* methylation. Also in these families transcriptional read-through correlates with subsequent promoter methylation. Gene-silencing by transcriptional read-through of a neighboring gene as demonstrated here in sense direction for *MSH2*, has been described earlier in antisense direction for *HBA2* in a patient with alpha-thalassemia and thus could represent a general mutational mechanism. Depending on the expression pattern of the neighboring gene that lacks its normal polyadenylation signal, this may cause either generalized or mosaic patterns of epigenetic inactivation, that are inherited over generations. Moreover, abrogation of polyadenylation signals due to chromosomal aberrations in cancer cells may result in aberrant promoter methylation and inactivation of tumor suppressor genes.

## S13.3

**Functional mechanism of genomic imprinting**

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## S14.1

**The P53 pathway acts to delay *in-vivo* senescence and aging**

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Cellular senescence of cultured cells relies heavily on activation of the p19<sup>Arf</sup>-p53 tumor suppressor pathway. This together with the observation that p19<sup>Arf</sup> expression increases with age in many tissues of humans and rodents, led to speculation that p53 activity drives *in vivo* senescence and natural aging. However, it has been difficult to experimentally test this hypothesis *in vivo* using a model system, because inactivation of p19<sup>Arf</sup> or p53 in mice results in early death from tumors with 100% penetrance. One approach to test the role of the p53 pathway in organismal aging would be to inactivate p19<sup>Arf</sup> or p53 in a mouse model that develops age-related pathologies at a young age. BubR1 insufficient mice (BubR1 hypomorphic), which die five times faster than wild-type mice, develop a variety of early-aging associated phenotypes including cachetic dwarfism, skeletal muscle atrophy

(sarcopenia), cataracts, arterial stiffening, loss of subcutaneous fat, reduced stress tolerance and impaired wound healing. We show that BubR1 hypomorphic mice exhibit high expression of p19<sup>Arf</sup> in skeletal muscle and fat. Surprisingly, inactivation of p19<sup>Arf</sup> exacerbates *in vivo* senescence and aging specifically in these tissues, suggesting that p19<sup>Arf</sup> is an attenuator, rather than an effector of aging and senescence. Furthermore, in accordance with a generalized anti-aging effect of the p53 pathway, we find that abrogation of p53 also selectively accelerates senescence and aging in skeletal muscle and fat of BubR1 hypomorphic mice. Importantly, BubR1 hypomorphic mice lacking the cell cycle inhibitor p21, a key target of p53 activation, exhibit the same accelerated aging characteristics and senescent cell accumulation as BubR1 mutants lacking p19<sup>Arf</sup> or p53, suggesting that p53 exerts its anti-aging effect through p21, and not through targets that mediate apoptosis. Based on these data, we propose that chronic stress associated with BubR1 insufficiency or normal aging, induces a relatively mild p53 transcriptional response that instead of promoting aging and senescence acts to attenuate aging by providing stress resistance.

## S14.2

**Aging and Cancer: Rival Demons?**

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Age is the largest single risk factor for developing cancer. In this regard, cancer is no different from a host of other age-related diseases -- cardiovascular disease, osteoporosis, neurodegeneration, etc. Nonetheless, cancer appears to differ from other age-related pathologies in conspicuous ways. First, cancer is dominated by hyperproliferation, rather than degeneration. Second, somatic are an essential driving force behind cancer development, but it is not clear whether is the case for other aging phenotypes. Third, cancer arises from renewable tissues, which promote longevity by allowing repair and regeneration, but are perennially and inherently at risk for developing malignant tumors due to mutations that can arise during DNA replication. Nonetheless, mutations are known to accumulate very early in life, and it is now clear that mutations alone are rarely enough to fully drive malignant tumorigenesis. How then do fundamental aging processes set the stage for the development of cancer, and do these processes differ from those that set the stage for other age-related pathologies? We present a model and supporting data to suggest that evolutionarily conserved tumor suppressive pathways, which evolved to protect complex multicellular organisms from cancer, can have deleterious late-life effects. We propose that the evolutionary antagonistic pleiotropy of certain tumor suppressor responses can drive aging phenotypes, including, ironically, late-life cancer. We suggest that these deleterious effects arise, in part, from the transcriptional response of normal cells to damage and other potentially oncogenic insults, which leads to the secretion of factors that can alter normal tissue structure and function, and ultimately create a tissue milieu that is conducive for the development of cancer. This model offers a new paradigm for understanding both aging and cancer phenotypes, and new possibilities for ameliorating the deleterious effects of certain tumor suppressive strategies.

## S14.3

**Insulin signalling, ageing and age-related disease**

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Although aging appears to be stochastic in nature, involving accumulation of molecular damage caused by such processes as oxidation or glycation, the rate of ageing is also influenced by genetic variation. For example, there are striking differences in longevity between animal species and mutations in single genes can extend lifespan in laboratory animals. There is growing evidence that the insulin/insulin-like growth factor (IGF) signalling (IIS) pathway, which has long been known to play pleiotropic roles in the development, growth, reproduction, stress resistance and metabolism of multicellular animals, is a key evolutionarily conserved regulator of longevity. The pleiotropic effects of IIS upon organismal physiology are largely mediated by intracellular signalling adaptor proteins the best characterised of which are the insulin receptor substrate (IRS) proteins. We recently undertook a systematic analysis of the role of IRS proteins in mammalian lifespan. Fe-

male *Irs1*<sup>-/-</sup> mice were long-lived and, importantly, resistant to a range of age-related pathologies, including skin, bone, immune and motor dysfunction. These improvements in health were seen despite mild, life-long insulin resistance. Thus, enhanced insulin sensitivity is not a prerequisite for IIS mutant longevity. *Irs1*<sup>-/-</sup> female mice also displayed normal anterior pituitary function distinguishing them from long-lived somatotrophic axis mutants. In contrast, *Irs2*<sup>-/-</sup> mice were short-lived, while *Irs1*<sup>-/-</sup> and *Irs2*<sup>-/-</sup> and *Irs1*<sup>-/-</sup>:*Irs2*<sup>-/-</sup> mice showed normal lifespan. We are currently exploring potential downstream effector pathways that mediate the longevity assurance mechanisms regulated by IRS signalling and the role of this signalling pathway in neurodegenerative disease. IRS signalling is a key intracellular determinant of age-related health, and hence a potential point of intervention for broad-spectrum, preventative therapy against age-related diseases.

#### S15.1

##### **Vangl genes and neural tube defects**

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Neural tube defects (NTDs) are very frequent congenital abnormalities in humans. Recently, we have documented independent association of *Vangl1* and *Vangl2* gene mutations with NTDs. In the *Looptail* mouse, homozygosity (but not heterozygosity) for loss-of-function alleles at *Vangl2* causes the severe NTD craniorachischisis, while heterozygosity for mutant variants of *VANGL1* is associated with NTDs in a human cohort of sporadic and familial cases. Recent functional studies of *Vangl2* *Looptail* alleles (S464N, D255E) show that loss-of-function in these two variants is associated with reduced protein stability and absence of mature protein at the membrane. To further understand the role of *Vangl1* in normal development, we created a mouse mutant with an inactivating mutation at *Vangl1* (*Vangl1*<sup>gt</sup>). *Vangl1* shows a dynamic pattern of expression in the developing neural tube and notochord at the time of neural tube closure. *Vangl1*<sup>gt/+</sup> heterozygotes and *Vangl1*<sup>gt/gt</sup> homozygotes are viable and fertile, although *Vangl1*<sup>gt/gt</sup> display subtle alterations in polarity of inner hair cells of the cochlea. Remarkably, and as opposed to healthy *Vangl1*<sup>gt/+</sup> and *Vangl2*<sup>gt/+</sup> heterozygotes, *Vangl1*<sup>gt/+</sup>; *Vangl2*<sup>gt/+</sup> double heterozygotes show profound develop-

mental defects that include severe craniorachischisis, inner ear defects (misorganization of the stereociliary bundles of hair cells of the organ of Corti), and cardiac abnormality (aberrant right subclavian artery). These results show that genetic interaction between *Vangl1* and *Vangl2* genes causes neural tube defects. They raise the possibility that interaction between individual *Vangl* genes and other genetic loci and/or environmental factors may additionally contribute to the etiology of NTDs.

#### S15.2

##### **The ciliopathies: a model for dissecting context-dependent pathogenesis**

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No abstract received as per date of printing. Please see [www.eshg.org/eshg2009](http://www.eshg.org/eshg2009) for updates.

#### S15.3

##### **Graded Sonic Hedgehog Signaling and the Control of Neuronal Subtype Identity in Vertebrate Embryos**

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Neuronal subtype specification in the vertebrate neural tube is one of the best-studied examples of embryonic pattern formation. Distinct neuronal subtypes are generated in a precise spatial order from progenitor cells according to their location along the anterior-posterior and dorsal-ventral axes. Underpinning this organization is a complex network of multiple extrinsic and intrinsic factors. In ventral regions, the secreted protein Sonic Hedgehog (Shh) acts in graded fashion to organize the pattern of neurogenesis. This is a dynamic process in which increasing concentrations and durations of exposure to Shh generate neurons with successively more ventral identities. Interactions between the receiving cells and the graded signal underpin the mechanism of Shh action. In particular the transcriptional regulation of genes induced or repressed by Shh signaling plays an essential role in shaping the graded readout. Thus the accurate patterning of the neural tube relies on the continuous processing and constant refinement of the cellular response to graded Shh signaling.

**C01.1****mRNA-Seq Whole Transcriptome Analysis of a Single Cell**

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We developed a digital gene expression profiling assay at single cell resolution by combining a modified single cell whole transcriptome amplification method with the next generation sequencing technique, SOLiD™ System. Using only a single mouse blastomere, our mRNA-Seq assay can detect the expression of 74% (5,270) more genes than microarray techniques. Moreover, 8 - 19 % of the genes with multiple known transcript isoforms express at least two isoforms in the same blastomere or mature oocyte, which unambiguously demonstrated the complexity of the transcript variants at whole genome scale. Finally, for Dicer and Ago2 knockout oocytes, we also showed that in Dicer knockout and Ago2 knockout mature oocytes, 1,924 and 1,687 genes respectively were abnormally upregulated, and 1,343 and 987 transcripts respectively were downregulated compared to wildtype controls, which proves the global importance of small RNAs (including microRNAs and endogenous siRNAs) for oogenesis. The main technical novelty of this work is the combination of an improved unbiased amplification of cDNAs from single cells with well over a 100 million reads, or a few gigabases of cDNAs on SOLID™. This not only allowed us to discover many novel transcripts that have been overlooked but also to get a quantitative estimate of their abundance in the cell by the frequency with which the sequence occurs in the mRNA-Seq reads. This single cell mRNA-Seq assay will greatly enhance our ability to analyze transcriptome complexity during mammalian development, especially for early embryonic development and for stem cells.

**C01.2****Integrated analysis of high-resolution transcriptomics data reveals new insights into the differentiation state-dependent control of transcript isoform abundance**

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Around 90% of human genes have been estimated to undergo alternative splicing. Apart from switching genes on and off, switching between transcript isoforms can be used for fine-tuning and orchestration of cellular differentiation. Using the myoblast cell line C2C12 as a well-controlled model for cell differentiation, we applied a variety of high-resolution genomics technologies to study gene transcription in undifferentiated and differentiated cells. We applied CAGE-Seq (cap analysis of gene expression followed by Illumina deep sequencing) to quantitatively identify the 5'-ends of transcripts, SAGE-Seq to quantify the 3'-ends of transcripts, and assayed mRNA degradation rates on Affymetrix exon arrays. We found 1400 transcription start sites not previously annotated. Around 50% of the expressed genes demonstrated use of multiple polyadenylation sites. We observed extensive qualitative and quantitative differences in use of transcription start sites, internal exons, 3'-UTRs, and polyadenylation sites between differentiated myotubes and undifferentiated myoblasts. Splice variants from some genes were produced at comparable levels, but degraded with different efficiencies; a transcript from the *Itga7* gene with an additional internal exon was much more abundant in differentiated than in undifferentiated cells, mainly because of specific and extremely rapid degradation of transcripts lacking this exon. Since it is thought that the abundance of different splice isoforms is mainly controlled by tissue-specific splicing factors, this represents a new mechanism to regulate the ratio between different splice isoforms. We conclude that promoter usage, alternative splicing and RNA degradation must be tightly coupled through yet unknown mechanisms.

**C01.3****Genomic variation detection by DNA selection and high throughput sequencing**

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The resequencing of a targeted region of the genome has become a major goal in order to understand the correlation between genomic and phenotypic variability. We have optimized a genomic selection method for high throughput sequencing. The repeat-masked contiguous region of 8.9Mb was targeted on human chromosomes 21 and 7. We used DNA from an individual from the International HapMap Project for which the genotype data are available. After microarray-based enrichment and sequencing of genomic DNA from chromosome 21 we observed a 260-fold enrichment with 41% of reads from the targeted region. The median coverage of the targeted region using two lines of an Illumina GA2 sequencing instrument was 16-fold. We also observed that regions with low sequence coverage are AT rich and are close to low-complexity DNA stretches. 83% of SNPs have at least 4-fold coverage, and 80% of the SNPs identified were already listed in dbSNP. For these dbSNP positions our sequence genotypes are concordant in 92% of cases with previously obtained genotypes of NA12872. 54% of all dbSNP positions had at least 15-fold sequence coverage, the coverage previously estimated as minimal for rigorous SNP search. At this threshold, 98.8% of dbSNP genotypes are concordant between sequencing and HapMap data for NA12872. Validation experiments using Sanger sequencing after PCR amplification were done for 46 SNPs covered 15-20 fold; the confirmation rate obtained was 96%.

We conclude that DNA selection method could provide an accurate and inexpensive search for genomic variability.

**C01.4****Adult human brain samples deep sequencing of small-RNAs reveals specific expression profiles in different brain areas**

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Small RNAs are non-coding RNAs of 20-30 nt in length, associated with members of the Argonaute family of proteins. Small RNAs are involved in the guidance of diverse types of gene regulation, typically resulting in reduced expression of target genes. In the central nervous system miRNAs are key in developmental processes, contributing to neuronal cell identity and synapse formation. miRNAs also play a role in mature neurons, participating in synaptic plasticity and neuronal survival. Alterations in miRNA-related pathways have been associated to several neurological and neurodegenerative diseases. Here we have used Illumina/Solexa deep sequencing to extensively characterize and profile small RNA libraries of three adult brain areas: frontal cortex, striatum and amygdala. In all the samples the majority of reads corresponded to previously annotated miRNAs. The most abundant sequences in all libraries included members of the let-7 family, mir-29a, mir-1, mir-101 and mir-103 miRNAs. Selective miRNAs were specifically enriched in each brain area. We have found strong variability in the mature miRNA reference sequence, mainly in the form of length modifications, that match the precursor sequence of the miRNA. Variability was also detected as nucleotide substitutions in the different positions of the reference mature miRNA, which was clearly reduced in the 5'-seed region. These results suggest a miRNA-signature in the different brain areas that may be related with the maintenance of the transcriptome in each brain structure. The present results further highlight the possible importance of the modified mature miRNA sequences in the physiology and pathology of the adult brain.

**C01.5****Estimation of MUTYH variant frequencies in pooled DNA with massive parallel sequencing**

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To evaluate the suitability of massive parallel sequencing by Illumina/Solexa sequencing technology for variant detection and allele frequency estimation, we sequenced the MUTYH gene in two pools of DNA (from breast cancer patients). A 6 kb long-range PCR (LRP) was designed containing exons 2-16. One pool consisted of 287 genomic DNA samples, serving as template for LRP. The second pool consisted of 88 LRP-products derived from individual samples. Equimolarity of the constituent samples was calculated from concentration measurements with fluorimetry for genomic DNA and high resolution melting curve analysis (HR-MCA) for LRP-products. Illumina sequencing results were compared to Sanger sequencing results of individual samples. Correlation between allele frequencies detected by both methods seemed poor in the first pool, probably due to variable DNA quality among samples, a too large pool size and unequal amplification caused by an Alu insertion polymorphism. Frequencies correlated well in the second pool, which allowed reliable detection of a frequency of 2 in 176 alleles (1.1%) or higher, whereas 2 of the 5 singletons detected by Sanger were significantly above background noise in the Illumina output. These results provide directions in designing high-throughput analyses of candidate genes in large series of patients and controls.

**C01.6****Next-Generation-Sequencing as a promising diagnostic tool in heterogeneous genetic conditions: the example of Hypertrophic Cardiomyopathy**

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Hypertrophic Cardiomyopathy (HCM) is the most common inherited cardiac disorder with a remarkable genetic and allelic heterogeneity (> 450 mutations in at least 20 genes). Molecular testing for HCM has a growing impact on the medical management of patients/families. To overcome the extensive genetic heterogeneity we had developed a microarray to resequence 30 Kbp including all exons (n=160), splice-sites and 5'-UTR of 12 HCM genes (HCM-custom-DNA-resequencing-array, HCM-RA, Fokstuen et al, 2008). This approach, now used in diagnostics, is very efficient and cheap but does not detect small indels (~14% of known HCM mutations). Moreover, HCM-RA lacks flexibility since gene additions require a redesign.

We now present new results obtained using short read next-generation-sequencing (NGS) of 12 HCM genes (targeted by multi-exonic amplicons) and a new downstream data analysis pipeline. DNAs from 19 patients (11 without known mutations, 8 positive-controls as a composite-pool) were assessed in one channel (exp.A). In another channel (exp.B), we sequenced a single patient with no known mutation. Mutations were confirmed by classical sequencing. In Exp.A, all the 8 known pathogenic mutations and SNPs previously identified were also found by NGS. Furthermore we identified novel variants: two indels and a nonsense mutation in MYBPC3 gene that likely cause the disease. In Exp.B, no pathogenic mutation but 140 non-coding dbSNPs were identified.

Although improvements are needed in target enrichment and data analysis, NGS holds considerable promises in high-throughput analysis of mutations/variants underlying the highly heterogeneous or multi-genic genetic disorders in clinical practice.

**C02.1****Phenotypic and genomic evaluation of 52 subjects with a Smith-Magenis-like phenotype: identification of new syndromic regions associated with altered gene dosage**

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Haploinsufficiency of the retinoic acid induced 1 gene (RAI1) results in Smith-Magenis syndrome (SMS), which includes developmental delays, sleep disturbance, self-injurious behaviors, and dysmorphic features. We evaluated 52 individuals referred for molecular analysis due to a possible SMS diagnosis. Screening for 17p11.2 deletions and RAI1 mutations was negative, suggesting that at least one other locus is responsible for the SMS-like phenotype. This cohort is clinically indistinguishable from SMS, with >90% overlap with core features, including developmental delays, sleep disturbance, self-injurious behaviors, motor dysfunction, obesity, and behavioral anomalies of the same type and prevalence as seen in SMS. We performed whole genome array CGH to identify genomic lesions contributing to the SMS-like phenotypes and identified 15 CNVs/52 cases, including 10 deletions and 5 duplications. These CNVs include novel genomic regions where altered gene dosage has not been previously associated with any clinical syndrome. We also identified several well-characterized regions associated with known syndromes, suggesting a need for better clinical phenotyping and discrimination between syndromes, as well as possible syndromic associations not previously appreciated. Genes in these regions contribute to development, neurological function, and behavior, all of which are affected in SMS. Further, some of these loci have been associated with autism, schizophrenia, and mental retardation, suggesting possible genetic heterogeneity. Given the phenotypic overlap between the SMS and SMS-like cases, these molecular data may provide insights into the function of RAI1 and may improve diagnosis, understanding, and potentially treatment of these complex behavior and mental retardation syndromes.

**C02.2****Further delineation of the 15q13.3 microdeletion and duplication syndromes: A clinical spectrum varying from non-pathogenic to a severe outcome**

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Recently, recurrent 15q13.3 micro-deletions were identified with identical proximal (BP4) and distal (BP5) breakpoints and associated with mild to moderate mental retardation and epilepsy. To further assess the clinical implications of this novel 15q13.3 micro-deletion syndrome 18 new probands and 18 family members with this deletion were molecularly and clinically characterised. Moreover, four patients with a duplication were studied.

The 15q13.3 micro-deletion caused a clinical spectrum ranging from normal development, to learning problems, to mild and even severe mental retardation. Remarkably, at least 11 of the 18 deletions identified were inherited from a normal parent. Moreover, seven out of ten tested sibs had the same deletion of which one had a mild de-

velopmental delay, four had only learning problems during childhood, whereas the other two had no learning problems at all. Unlike previous reports, seizures were not a common feature in our series (6%). Three patients had a cardiac defect (8%) and based upon earlier mice knockdown studies we suggest *KLF13* to be an interesting candidate gene underlying these heart anomalies. Overall, our findings broaden the phenotypic spectrum associated with 15q13.3 deletions and suggest that, in some individuals, deletion of 15q13.3 is not sufficient to cause disease.

Psychiatric disease was noted in two of four duplication patients, although these patients did not share a recognisable phenotype.

### C02.3

#### Incomplete penetrance and variable expressivity in a series of 11 French patients with 15q13.3 recurrent microdeletion detected using array-CGH.

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Since the large implementation of array-CGH in the diagnostic work-up of mental retardation, novel microdeletion syndromes have been described. In particular, the 15q13.3 microdeletion has been identified in 0.2-0.3% of individuals with mental retardation and epilepsy, schizophrenia, autism and other neuropsychiatric features. The critical region between BP4 and BP5 contains at least seven genes, including *CHRNA7*, which is considered a good candidate gene for the epilepsy phenotype. We report a series of 11 patients (8 index cases and 3 affected parents) presenting a developmental delay and a 1.5 Mb 15q13.3 recurrent microdeletion ascertained through 11 French CGH-array platforms. All 8 index cases presented mild to moderate mental retardation with absent or non-specific dysmorphic features. Only two had seizures, 4 had an abnormal electroencephalogram and none had an autistic behaviour. One patient presented a highly different phenotype, including hydrocephaly, joint dislocations, congenital lymphoedema and notable dysmorphic features associated to mental retardation. Familial studies could be performed in 5/8 index patients. Interestingly, all 15q13.3 microdeletions were inherited (3 from the mother, 2 from the father). The affected parents had mild mental retardation with epilepsy in one. In particular, the microdeletion was found in 2 completely asymptomatic mothers. This study is in favour of incomplete penetrance and more variable clinical expressivity than previously published. Therefore, the 15q13.3 recurrent microdeletion might be only considered as a risk factor for mental retardation. The search for mutations on the second allele of the *CHRNA7* gene is in progress in order to explain this incomplete penetrance.

### C02.4

#### Interstitial 18q21 microdeletions and a microduplication including the TCF4 gene causing Pitt Hopkins syndrome

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Deletions of chromosome 18q are relatively common. The majority of patients carry a terminal 18q deletion, also known as De Grouchy syndrome whereas interstitial 18q deletions have been detected in only a small number of patients. The introduction of high-resolution molecular techniques like array CGH allows for the detection of submicroscopic aberrations, leading to an increasing number of patients identified with small (interstitial) 18q deletions.

We have identified a (sub)microscopic 18q21 deletion ranging from 350 kb to 11 Mb in six sporadic patients with psychomotor retardation. In all patients the deleted region included TCF4, the gene associated with Pitt Hopkins syndrome (PHS). Our patients, however, did not show the classical PHS phenotype including the intermittent hyperventilation and facial gestalt as displayed by recently described patients with TCF4 mutations.

In addition, in a severely mentally retarded female patient with a 1 Mb duplication including the TCF4 gene was identified.

We conclude that deletions of the TCF4 gene are not always associated with classical PHS. Although the majority of patients with a microdeletion in this region show severe developmental delay, a clear genotype-phenotype correlation is lacking. Furthermore, duplications of the TCF4 region appear to be rare, leading to a phenotype which remains to be defined.

### C02.5

#### Another new microdeletion syndrome due to 11q13.2q13.4 cryptic deletion mediated by segmental duplications.

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By array-CGH, we identified a cryptic deletion of about 3.4 Mb, involving the chromosomal region 11q13.2-q13.4, in a child with psychomotor retardation and speech delay. At the breakpoints we found several highly homologous segmental duplications that could have mediated the imbalance through the well studied mechanism of non allelic homologous recombination (NAHR), supporting genomic instability in this region that might lead to recurrent chromosomal rearrangements and diseases. To our knowledge, this is the first report of an interstitial deletion extending from 11q13.2 to 11q13.4: most of the deletions reported on chromosome 11 involve the distal portion of the long arm and are originated by the presence of fragile sites or by imprinting mechanisms. Here we demonstrate that proximal 11q deletions might be generated through NAHR and might account as recurrent rearrangements leading to new genomic disorders. Moreover, the detection of an overlapping, slightly larger deletion in a second patient who shares several dysmorphic features with the first one, shows that the haploinsufficiency of this region determines a new clinically defined syndrome mainly characterized by preauricular tag, small low-set ears, tubular somewhat beaked nose with round overhanging tip and hypoplastic nares, short philtrum, small mouth, moderate severe developmental delay, and severe language delay.

### C02.6

#### Retrospective external quality assessment: the french ACLF online experience

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The External Quality Assessment (EQA) in cytogenetics was undertaken in France since 2005 and the ACLF (Association des Cytogénéticiens de Langue Française) has set up a computerised database for a constitutional retrospective EQA and a prospective EQA in onco-haematology (Groupe Français Cytogénétique Hémato-Oncologique) (<http://www.eaclf.org/infoCQE>).

The purpose of the retrospective EQA is to assess the technical quality of the laboratories and the biological and clinical interpretation of the results. The participant laboratories submit, online, reports concluded in the previous year, with three corresponding pictures of the karyotypes. All the reports are anonymised for the patient's name and the laboratory origin. The database enables a rapid, and secured handling of the assessed reports and pictures. The assessors are licensed cytogeneticist divided into 5 groups. They examine the reports and transmit back their conclusions to the database. The final results are saved and delivered anonymously by mail. A total of 50/70 laboratories participate in prenatal or/and postnatal scheme. In 2007, 131 postnatal reports and 181 prenatal (131 amniotic fluids + 50 chorionic villi) reports were uploaded. This represents 934 evaluated mitosis. The results showed that 74,3% of the reports had 3/3 correct classified karyotypes in R and/or G banding. The participants evaluated correctly the resolution level in 76,1% mitosis. Interpretation of the cases was correct in 94,87% but ISCN nomenclature was wrong in 26.7% reports. These results will help in improving the practice and quality in cytogenetic laboratories (accredited or submitting for accreditation) and to the accreditation of the test itself.

### C03.1

#### Joubert syndrome and related cerebellar disorders among Egyptian patients: Clinical and genetic heterogeneity

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Joubert syndrome and related cerebellar disorders (JSRD) are a group of autosomal recessive conditions characterized by hypotonia, ataxia, dysregulated breathing rhythms, oculomotor apraxia, intellectual disabilities and the unique pathognomonic finding "molar-tooth sign" (MTS) on brain imaging. There is a pleiotropic phenotypic presentation with various organs involvement including retina, kidney and liver, along with polydactyly and facial dysmorphisms explaining the genetic heterogeneity. We present 54 patients with JSRD derived from 34 families. Consanguinity was positive in 88.8% of cases. Their age ranged from 3 months to 38 years old. Based on the previous proposed classification of JSRD into 4 major subtypes, our patients were categorized as classic Joubert syndrome in 32 families, COACH syndrome in 5 families, CORS in one family and OFD IV in 2 families. Linkage analysis was done to NPHP1, AHI1, CEP290, TMEM67, RPGRIP1L, ARL13B and CC2D2A. Four families family were linked to one of the four known genetic loci; three of them showed novel AHI1 mutations (JBTS3) and a single family to JBTS1. Mutation in the INPP5E gene, encoding inositol polyphosphate-5-phosphatase E (INPP5E), which hydrolyzes the 5-phosphate of phosphoinositides PtdIns(3,4,5)P3 and PtdIns(4,5)P2 has been recently identified in a single family. INPP5E localized to cilia in the major organs affected in JS and its mutations promoted destabilization of cilia in response to stimulation. The rest of families were excluded from known and recently identified loci and are currently subjected to genome-wide linkage scans to identify novel loci. This work shows clearly clinical and genetic heterogeneity of Joubert syndrome.

### C03.2

#### CC2D2A mutations in Meckel and Joubert syndromes, a strong genotype phenotype correlation

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Meckel syndrome (MKS) is a lethal polymalformation syndrome characterised by cystic kidneys, polydactyly, a bile duct proliferation of liver, and a brain malformation, mainly an occipital encephalocele. Joubert syndrome (JS) is a neurological disorder, characterized by neurological signs ascribed to a cerebellar vermis hypoplasia and a characteristic "molar tooth sign" on brain axial images. Other variable features define the cerebello-oculo-renal syndrome (CORS). Allelism of Joubert and Meckel syndromes has been described at 3 loci : TMEM67/MKS3, CEP290/MKS4, RPGRIP1L/MKS5. Very recently a same homozygous splice mutation in the CC2D2A gene was reported in Finnish fetuses with MKS defining the 6<sup>th</sup> MKS locus. Short after, CC2D2A mutations in Joubert patients were reported (JBTS9).

Here we report the mutation and expression analysis of the CC2D2A gene during human development. We identified mutations in 11 MKS cases, of various ethnic origin, all predicting truncating alleles. These results confirm the involvement of CC2D2A in MKS and show its major contribution to the disease. We also identified missense CC2D2A mutations in two JS cases. Our data suggest a strong phenotype genotype correlation as null alleles were found in MKS while missense/hypomorphic mutations in at least one allele were found in all but one Joubert cases to date.

### C03.3

#### CA8 mutations cause a novel syndrome characterized by ataxia and mild mental retardation with predisposition to quadrupedal gait

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We describe a consanguineous Iraqi family in whom affected siblings had mild mental retardation and congenital ataxia characterized by quadrupedal gait. Genome-wide linkage analysis identified a 5.8 Mb interval on chromosome 8q with shared homozygosity among the affected persons. Sequencing of genes contained in the interval revealed a homozygous mutation, S100P, in carbonic anhydrase related protein 8 (CA8), which is highly expressed in cerebellar Purkinje cells and influences inositol triphosphate (ITP) binding to its receptor ITPR1 on the endoplasmatic reticulum and thereby modulates calcium signaling. We demonstrate that the mutation S100P is associated with proteasome-mediated degradation, and thus presumably represents a null mutation comparable to the Ca8 mutation underlying the previously described waddles mouse, which exhibits ataxia and appendicular dystonia without pathological abnormalities of either the central or the peripheral nervous systems. Subsequently, we identified the mutation R237Q in a highly conserved region of CA8 in an unrelated Iranian family with mild mental retardation and ataxia without quadrupedal gait. Magnetic resonance imaging studies of an affected person revealed no structural cerebral or cerebellar abnormalities. Our findings underline the importance of ITP-mediated signaling in cerebellar function and provide suggestive evidence that congenital ataxia paired with cerebral dysfunction may, together with unknown contextual factors during development, predispose to quadrupedal gait in humans.

### C03.4

#### Search for genes implicated in new forms of recessive ataxia

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Autosomal recessive cerebellar ataxias are a heterogeneous group of neurological disorders characterized by the degeneration or abnormal

development of the cerebellum and/or the spinal cord, and, in most cases, by onset before the age of 20 years. Causative genes have been identified for the most frequent forms of autosomal recessive ataxia. Nonetheless, genes remain unidentified for about half of the cases.

A SNP-based genome-wide scan in a consanguineous family with 3 affected siblings allowed us to identify a novel locus for autosomal recessive ataxia on chromosome 3qter. By direct sequencing of genes in this family, we have identified a homozygous frameshift mutation shared by the patients and heterozygous or absent from the 5 healthy siblings. The patients have an early onset of ataxia before 7 years associated with delayed motor development, dysarthria, epilepsy with no relapse observed after treatment since 3 years of age. Two of the patients present also mental retardation. Nerve conduction studies revealed no evidence of associated peripheral neuropathy. Bioinformatics predictions show that the homologs of the mutant gene belong to a subfamily of genes coding for Plekstrin homology domain. A close plekstrin homolog may be linked to small GTPase signaling and colocalizes with late endosomal/lysosomal vesicles in osteoclast-like cells, suggesting a putative function in vesicular transport in the osteoclasts. These data suggest that our recessive ataxia gene is involved in membrane signaling, a function that can now be tested by molecular studies in order to understand the mechanism of this novel form of recessive ataxia.

#### C03.5

#### *tRNA Splicing Endonuclease mutations cause Pontocerebellar Hypoplasia*

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Pontocerebellar hypoplasia (PCH) represents a group of neurodegenerative autosomal recessive disorders with prenatal onset (PCH1-6). Children suffer from severe mental and motor impairments due to atrophy or hypoplasia of the cerebellum, hypoplasia of the ventral pons, microcephaly and variable neocortical atrophy. The disease is progressive and usually patients die before they reach adulthood.

We identified a common mutation in TSEN54 (A307S) in the majority of European PCH2 patients. TSEN54 is one of the four subunits of the tRNA splicing endonuclease (TSEN34, TSEN2 and TSEN15). The TSEN complex is responsible for the splicing of intron containing tRNAs and also plays a role in pre-mRNA 3'end formation. In PCH patients without the A307S mutation, we identified other missense and nonsense mutations in TSEN54, TSEN34 and TSEN2 subunits.

In situ hybridization for TSEN54 using LNA/2OME probes revealed that TSEN54 is highly expressed in neurons of the pons, cerebellar dentate and olfactory nuclei.

Northern blot analysis of tRNA-Tyrosine from fibroblasts of 3 patients did not show unspliced products.

The molecular mechanism behind pontocerebellar hypoplasia remains unclear. In order to study the mechanisms underlying PCH we performed knock down experiments in zebrafish. Injection of TSEN54 or TSEN2 morpholino oligonucleotides in zebrafish embryos results in similar neurodevelopmental phenotypes, most prominently affecting brain development.

#### C03.6

#### *Retinal neurone remodelling induced by polyglutamine toxicity in a SCA7 mouse model*

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Spinocerebellar ataxia type 7 (SCA7) belongs to a group of nine inherited neurodegenerative disorders caused by polyglutamine (polyQ)

expansion in unrelated disease proteins. PolyQ expansion confers to mutant proteins a toxic gain of function, which compromises neuronal function and survival. However, the molecular and cellular mechanisms underlying polyQ expansion toxicity remain unclear.

SCA7 is unique among polyQ diseases to cause retinal degeneration and blindness. To get insight into the mechanisms of polyQ toxicity, we are studying the SCA7 retinopathy in the R7E transgenic mice, which express the polyQ expanded ataxin-7 under the rhodopsin promoter. Initial studies showed that R7E retinopathy was characterized by a progressive reduction of photoreceptor segments and of electroretinograph (ERG) activities, however, without extensive loss of photoreceptors. That differed R7E retinopathy from other retinal degenerations in mammalian (inherited or light-induced), in which degenerative outer segment disappearance is typically followed by photoreceptor death. We now describe the morphological deconstruction of R7E photoreceptor cells, which is reminiscent of the structural reorganization that assures the survival of photoreceptors in some retinal detachment paradigms. Moreover, a subset of R7E photoreceptors migrate out of outer nuclear layer, die by non-apoptotic cell death and thereby cause a continuous loss of photoreceptor cells along the pathology. Remarkably, some photoreceptors expressing mutant ataxin-7 undergo cell division and likely contribute to maintain the photoreceptor cell population until late disease stage.

In conclusion, in response to ataxin-7 toxicity photoreceptors undergo a wide range of cell fate, including adaptative deconstruction, lethal migration and proliferative cell renewal.

#### C04.1

#### *Spondylocheiro dysplastic form of the Ehlers-Danlos Syndrome - A novel recessive entity caused by mutations in the zinc transporter gene SLC39A13*

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We present clinical, radiological, biochemical and genetic findings on six patients from two consanguineous families who show EDS-like features and radiological findings of a mild skeletal dysplasia. The EDS-like findings comprise hyperelastic, thin, and bruiseable skin; hypermobility of the small joints with a tendency to contractures; protuberant eyes with bluish sclerae; hands with finely wrinkled palms, atrophy of the thenar muscles and tapering fingers. The skeletal dysplasia comprises platyspondyly with moderate short stature, osteopenia, and widened metaphyses. Patients have an increased ratio of total urinary pyridinolines, lysyl pyridinoline/hydroxylysyl pyridinoline (LP/HP), of ~1 as opposed to ~6 in EDS VI or ~0.2 in controls. Lysyl and prolyl residues of collagens were underhydroxylated despite normal lysyl hydroxylase and prolyl 4-hydroxylase activities; underhydroxylation was a generalized process as shown by mass spectrometry of the  $\alpha 1(I)$ - and  $\alpha 2(I)$ -chain derived peptides of collagen type I and involved at least collagen types I and II. A genome-wide SNP-scan and sequence analyses identified in all patients a homozygous c.483\_491del9 mutation in SLC39A13 that encodes for a membrane-bound zinc transporter SLC39A13. We hypothesize that an increased Zn<sup>++</sup> content inside the endoplasmic reticulum competes with Fe<sup>++</sup>, a cofactor which is necessary for hydroxylation of lysyl and prolyl residues, and thus explains the biochemical findings. These data suggest a novel entity which we have designated "spondylocheiro dysplastic form of EDS (SCD-EDS)" to indicate a generalized skeletal dysplasia involving mainly the spine (spondylo) and striking clinical abnormalities of the hands (cheiro) in addition to the EDS-like features.

**C04.2****Frontorhiny, a distinctive presentation of frontonasal dysplasia caused by recessive mutations in the ALX3 homeobox gene**

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We describe a recessively inherited and distinctive frontonasal malformation characterized by hypertelorism, wide nasal bridge, short nasal ridge, bifid nasal tip, broad columella, widely separated slit-like nares, long philtrum with prominent bilateral swellings, and midline notch in the upper lip and alveolus. Additional recurrent features present in some individuals include ptosis and midline dermoid cysts of craniofacial structures. Assuming recessive inheritance we carried out autozygosity mapping and found linkage to chromosome 1p13.3 in 3 independent families. Three different homozygous pathogenic mutations were detected in the outstanding candidate gene in this region, the *aristaless*-related ALX homeobox 3 transcription factor, *ALX3*. Subsequently, we ascertained 4 more families with a further 4 different mutations. All of the mutations are predicted to lead to severe or complete loss of function and consisted of missense substitutions at critical positions within the conserved homeodomain, as well as non-sense, frameshift and splice site mutations. Our findings contrast with previous studies in the mouse, which showed no phenotype in *Alx3*<sup>-/-</sup> homozygotes, apparently owing to functional redundancy with the paralogous *Alx4* gene. However, we demonstrate that both *ALX3* and *ALX4* are expressed in the developing frontonasal region in human embryos. We conclude that *ALX3* is essential for normal facial development in humans and that deficiency causes a clinically recognisable phenotype, which we term frontorhiny.

**C04.3****ALX4 dysfunction disrupts craniofrontonasal and hair follicle development**

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Two families with a severe cranio-fronto-facio-nasal malformation syndrome characterized by frontonasal dysostosis, large cranial skull defects, associated with total alopecia, hypogonadism and cryptorchism were presented as a novel clinical entity at the last ESHG conference in Barcelona (Abstract No:C01.6). Using Affymetrix 250K SNP Array genotyping and a homozygosity mapping, we identified the locus for this entity on chromosome 11p11.2-q12.3. The *ALX4* gene was tested as a highly relevant candidate in the 19.8 Mb critical interval, and we found the homozygous nonsense mutation, p. R265X (c.793C>T), in affected individuals of both families. One heterozygous parent showed parietal foramina in the radiological evaluation. *Alx4* plays an important role in the development of structures derived from craniofacial mesenchyme, first branchial arch and the limb bud. Heterozygous *ALX4* mutations were described in patients with parietal foramina. To date, no homozygous *ALX4* mutations have been reported in human. RNA studies on primary fibroblast cell lines from our patients indicated that the mutated *ALX4* transcript is present and not degraded by nonsense-mediated mRNA decay. The mutation is predicted to cause a truncation of the *ALX4* protein within the functionally essential homeodomain, most likely leading to impairment of DNA binding. Histological and immunohistological analysis of patient's skin biopsy showed changes in

the epidermal architecture, rudimentary hair follicles, and significant changes in epidermal expression markers, indicating the essential role of *ALX4* also in skin structure and proper hair follicle development. This CRANIRARE consortium study is supported by Turkish Research Council(TUBITAK) as a partner of European Research Area Network

**C04.4****TRPS1, a regulator of chondrocyte proliferation and differentiation, interacts with the activator form of GLI3**

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The TRPS1 gene on human chromosome 8q24.1 encodes a multi zinc finger transcription factor protein. Mutations in TRPS1 cause the tricho-rhino-phalangeal syndrome (TRPS). Besides typical craniofacial anomalies, skeletal malformations are characteristic hallmarks of patients with TRPS.

Here we show that TRPS1 interacts with Indian hedgehog (Ihh)/GLI3 signalling and regulates chondrocyte differentiation and proliferation. By immunoprecipitation assays using transiently transfected cells as well as native tissue samples from embryonic mouse limbs, we could demonstrate that TRPS1/Trps1 specifically interacts with the activator form of GLI3/Gli3, whereas a direct binding of the repressor form of GLI3/Gli3 could be excluded. GST pull-down experiments were used to verify the interaction of the isolated GLI3 activator domain with TRPS1. Through the use of different truncated TRPS1 constructs, a domain of 185 aa, containing three predicted zinc fingers, was shown to be sufficient for the interaction with GLI3.

Using different mouse models we find that in distal chondrocytes Trps1 and the repressor activity of Gli3 are required to expand distal cells and locate the expression domain of Parathyroid hormone related peptid. In columnar proliferating chondrocytes Trps1 and Ihh/Gli signalling have an activating function. The differentiation of columnar and hypertrophic chondrocytes is supported by Trps1, independent of Gli3. Trps1 seems thus to organize chondrocyte differentiation interacting with different subsets of co-factors in distinct cell types.

**C04.5****Homozygous disruption of an extracellular matrix component cause Temtamy preaxial brachydactyly syndrome**

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The Temtamy preaxial brachydactyly syndrome (TPBS, OMIM 605282) is an autosomal recessively inherited congenital syndrome characterized by bilateral symmetrical preaxial brachydactyly and hyperphalangism of digits, multiple congenital anomalies, mental retardation, sensorineural deafness, and growth retardation. Skeletal anomalies in TPBS patients include progressive kyphoscoliosis and pectus excavatum.

We used a homozygosity mapping strategy in two consanguineous Egyptian families with TPBS, including the original family described by Temtamy, to map the TPBS locus. Linkage analysis with Affymetrix 250K SNP array in both families identified the locus on the long arm of chromosome 15. We tested several highly relevant positional candidate genes located within the 2 Mb critical region and found two homozygous causative mutations in one of these genes, named here *TPBS1*. A homozygous 1-bp deletion c.14delG in exon 1 was found in one family, while the index patient of the second family carried a homozygous 30-bp deletion, c.44\_73del30, also located in exon 1. Both mutations were not present in 120 healthy controls. The encoded protein is an extracellular matrix (ECM) component involved in the regulation of cartilage growth activity. We used a morpholino knockdown strategy in zebrafish for further functional characterization of the encoded protein. Currently, the observed developmental phenotype of these zebrafishes is analyzed in detail and will give novel insights into the pathophysiology of Temtamy preaxial brachydactyly syndrome.

**C04.6****Duplication of the EFNB1 gene in familial hypertelorism: imbalance in ephrin-b1 expression and abnormal phenotypes in humans and mice**

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Familial Hypertelorism, characterised by widely spaced eyes, classically shows autosomal dominant inheritance (Teebi type), but some pedigrees are compatible with X-linkage. No pathogenic mechanism has been described previously, but clinical similarity has been noted to craniofrontonasal syndrome (CFNS), which is caused by mutations in the X-linked EFNB1 gene.

Here we report a family in which females in three generations presented with hypertelorism, but lacked either craniosynostosis or a grooved nasal tip, excluding CFNS. DNA sequencing of EFNB1 was normal, but MLPA indicated a duplication of all 5 exons of EFNB1, which segregated with the hypertelorism. We characterised the duplication breakpoint sequence, revealing a direct duplication of 937 kb including EFNB1 and two flanking genes, PJA1 and STARD8. By use of Pyrosequencing, we measured imbalance of EFNB1 expression in the affected grandmother. After correction for skewed X-inactivation, we show that the X chromosome bearing the duplicated EFNB1 genes produces approximately twice as much EFNB1 transcript as the normal X chromosome.

We propose that in the context of X-inactivation, the difference in expression level of EFNB1 between the normal and duplicated X chromosomes results in abnormal cell sorting during embryogenesis, leading to hypertelorism. To support this hypothesis we provide evidence from a mouse model carrying a hypomorphic *Efnb1* allele, that abnormal cell sorting occurs in the cranial region. Hence we propose that X-linked cases resembling Teebi hypertelorism may have a similar pathogenesis to CFNS, and that cellular mosaicism for different levels of ephrin-b1 (as well as simple presence/absence) leads to craniofacial abnormalities.

**C05.1****Combined analysis of 19 common validated type 2 diabetes susceptibility gene variants show moderate discriminative value and no evidence of gene-gene interaction**

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**Background:** The list of validated type 2 diabetes susceptibility variants has recently been expanded from three to 19. The identified variants are common and have low penetrance in the general population. The aim of this study was to examine for gene-gene interactions and investigate the combined effect of the 19 variants by applying receiver operating characteristics (ROC) to demonstrate the discriminatory value between glucose-tolerant individuals and type 2 diabetes patients in a cross-sectional population of Danes.

**Methods:** The 19 variants were genotyped in three study populations: The population-based Inter99 study, the ADDITION study, and in additional type 2 diabetic patients and glucose-tolerant individuals. The case-control studies involved 4,093 type 2 diabetic patients and 5,302 glucose-tolerant individuals.

**Results:** Single variant analyses demonstrated allelic odds ratios (OR) ranging from 1.04 (95%CI: 0.98,1.11) to 1.33 (95%CI: 1.22,1.45). When combining the 19 variants subgroups with extreme risk profiles showed 3-fold difference in risk of type 2 diabetes (lower 10% carriers with <15 risk alleles vs. upper 10% carriers with >22 risk alleles, OR 2.93 (95%CI: 2.38,3.62,  $p=1.6\times 10^{-25}$ ). We calculated the area under a ROC curve to estimate the discrimination rate between glucose-toler-

ant individuals and type 2 diabetes patients based on the 19 variants. We found an area under the ROC curve of 0.60. Two-way gene-gene interaction showed few nominal interactions.

**Conclusion:** The 19 validated variants enables detection of subgroups in substantial increased risk of type 2 diabetes, however the discrimination between glucose tolerance and type 2 diabetes is still too inaccurate to achieve clinical value.

**C05.2****Joint re-analysis of twenty-nine correlated SNPs supports the role of PCLO/Piccolo as a causal risk factor for major depressive disorder**

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The first genome-wide association study (GWAS) for major depressive disorder (MDD) has implicated the pre-synaptic protein Piccolo, but results from multiple replication cohorts remained inconclusive. We propose a simple method for the joint (re-)analysis of multiple SNPs, based on published summary data. Our approach is based on two observations. Firstly, finemapping studies are focused, by design, on a limited number of moderately to strongly correlated SNPs. All tested SNPs are expected to reflect the true association of the unknown causal variant proportional to their LD with it, in concordance with the "Fundamental Theorem of the HapMap". Secondly, given such correlated SNP data it has been suggested before that a joint analysis of all markers together is most powerful for detecting a true association. A closer examination of the results reported in the GWAS study reveals that the data indeed concur with the "Theorem of the HapMap". Based on the above we re-analyzed the replication data using a novel joint test of association and conclude and the results strongly favors Piccolo to be a causal risk factor for major depression. This study was performed within the framework of Top Institute Pharma project: number T5-203.

**C05.3****Unified framework for epistasis detection in (un)relateds**

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When searching for epistatic patterns parametric regression approaches have severe limitations when there are too many independent variables in relation to the number of observed outcome events. Alternatively, the non-parametric Multifactor Dimensionality Reduction method, MDR (Ritchie et al. 2001), can be applied. The common feature of MDR and its extensions is that they are extremely computer-intensive, that best models are evaluated on the basis of cross-validation (prediction accuracy measures) and permutations and that only one such best model is proposed when looking at interactions of a particular order.

We propose a novel unified multifactor dimensionality reduction strategy for genetic interaction association analysis that can handle both unrelated individuals and families of any structure, different outcome types (e.g., categorical, continuous or survival type), easy covariate handling or adjustment for lower order interactions or confounding factors, all within the same framework. When applied to family data, we obtain a less computationally intensive method than current MDR adaptations to family-data and allow several clusters of markers to be proposed as showing significant association with the outcome under investigation. This better reflects locus heterogeneity and genetic heterogeneity, usually present in complex diseases.

Our epistasis detection method is further evaluated and validated via a simulation study, by computing type I error and power under a variety of scenarios, and via application to a real-life data set.

**C05.5****Development of molecular pathways analysis of GWAS data: application to schizophrenia and bipolar disorder**

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Genome-wide association studies (GWAS) provide substantially greater potential to detect common risk variants of modest effect in complex disorders than previous positional cloning methodologies. A major criticism has been that these studies have, to date, explained only a small fraction of predicted heritability despite large sample sizes and genome-wide SNP coverage. The joint action of common variants within pathways may play a major role in predisposing to complex genetic disorders, where multiple genes may contribute to susceptibility. Thus, there is a pressing need to develop more sophisticated mining techniques to identify biologically meaningful signals in data generated by GWAS. Molecular pathways- or systems-based approaches to mining complex GWAS are currently gaining prominence. Here we describe a novel SNP ratio test (SRT) that compares the observed to expected ratios of significant to non-significant SNPs within versus outside of pathways using GWAS data. In a recent GWAS we reported evidence that possibly thousands of common genetic variants of small effect contribute substantially to variance in both schizophrenia and bipolar disorder susceptibility. We now report a two-stage molecular pathways analysis testing 212 experimentally validated molecular pathways using discovery (International Schizophrenia Consortium (ISC); n=6,909) and validation (Genetic Association Information Network (GAIN); n=2,729) schizophrenia case-control samples. We also examine whether risk pathways identified by this method contribute to bipolar disorder susceptibility using the Wellcome Trust Case Control Consortium sample (n=4,847).

**C05.6****A Comparison of Methods for Testing Association Between Uncertain Genotypes and Quantitative Traits**

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Interpretability and power of genome wide association studies can be increased by imputing unobserved genotypes, using a reference panel of individuals genotyped at higher marker density. For many markers, genotypes cannot be imputed with complete certainty, and the uncertainty needs to be taken into account when testing for association with a given phenotype. In this paper we compare the statistical properties of currently available methods for testing association between uncertain genotypes and quantitative traits. We propose a new method that has superior performance, compared to previously available methods. Our new method is based on exact maximization of the likelihood function, and use a mixture model to accommodate non-normal trait distributions. Unlike some previously described methods, the proposed new method controls the false positive rate, without requiring ad hoc filtering rules, and harsh transformations of the trait under study. Furthermore, our in-silico analysis showed that the new method can increase power to detect associations when the trait is non-normally distributed. Its computation time is around one CPU-day for a genome wide scan, with 2.5M SNPs and 5,000 individuals, which is comparable to previously proposed methods. Finally, in a case study of three lipid phenotypes, we demonstrate that the superior statistical properties of the new method lead to an improved selection of SNPs that successfully replicate in independent samples.

**C06.1****Variants of the Xeroderma Pigmentosum Variant gene (POLH) are associated with melanoma risk**

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Background: Xeroderma pigmentosum variant (XPV) is a rare recessive autosomal genodermatosis predisposing to multiple early onset skin cancers, including melanoma. XPV results from mutations of the POLH gene that encodes a DNA translesion polymerase. In this work, we tested the hypothesis that POLH variants could be associated with melanoma risk.

Patients and methods: A common non-synonymous POLH variant, c.1783A>G p.M595V, was genotyped in 1075 melanoma patients and 1091 ethnic matched controls from France. In addition, we searched for rare POLH variants by sequencing the entire coding sequence in 201 patients having a familial history of melanoma (n= 123), sporadic multiple melanomas (n= 65) and a melanoma associated with a skin carcinoma (n= 13).

Results: Overall, the c.1783G, p.595V allele was statistically associated with melanoma (respective allelic frequencies, 0.040 versus 0.022, P-value=1.17x10-3, OR=1.86 [1.27-2.71]), which was further confirmed by a meta-analysis including 274 patients and 174 matched controls from Italy (P-value =7.7x10-4, OR =1.84 [1.29- 2.63]). Interestingly, three non-synonymous POLH variants were identified in 3 patients (c.295G>A p.V99M, c.815T>C p.I272T and c.1745C>T p.S582L) that were absent in 352 chromosome controls from healthy subjects.

Conclusion: Our data strongly suggest that POLH variants could act as low penetrance melanoma predisposing alleles. Hence, in addition to pigmentation genes, polymorphism of other genes implicated in UV response is associated with predisposition to melanoma. Replication studies in other populations are awaited to precise these data.

**C06.2****Identification of novel genes involved in colorectal cancer predisposition**

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Colorectal cancer (CRC) is the second most common cancer in the Western world in terms of both incidence and mortality rate. A positive family history of CRC is observed in about 25% of the cases. High-penetrant germline mutations in *APC*, *MUTYH* or the mismatch repair genes *MLH1*, *MSH2*, *MSH6* and *PMS2* account for less than 5% of hereditary cases whereas in the majority of these families the genetic defect is still unknown. In order to identify novel moderate- to high-risk mutations contributing to CRC predisposition we employed genome-wide copy number profiling using high-resolution SNP-based arrayCGH on normal tissue DNA from 32 early-onset patients with microsatellite-stable CRC without polyposis. We identified small (100-160kb) copy number anomalies in five independent families (16%), in all cases affecting only a single gene. None of the genes had previously been described to be involved in colorectal cancer susceptibility. All genomic lesions were validated with multiplex ligation-dependent probe amplification (MLPA). In four cases we were able to establish that the aberrations were inherited from one of the parents. Two of the genomic lesions were deletions affecting a microRNA gene, illustrating that constitutional defects in these gene expression regulators might be common. Interestingly, at least two of the identified genes could be linked to pathways involved in CRC development. In an ongoing locus-specific validation screen of independent families with suspected familial CRC (currently ~250), we thus far found at least one of the

genes to be recurrently affected, which strongly supports its role in CRC predisposition.

#### C06.3

##### TheTRIM8 gene is a novel player of p53 pathway

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p53 helps to maintain genomic integrity, regulates the cell-stress response, and controls human cancer development and progression. Approximately half of all human malignancies carry mutant p53, and many tumours containing wild-type p53 have abnormalities in p53 regulators.

By using a microarray based approach we found that p53 significantly induces the expression of TRIM8, a gene belonging to the Tripartite Motif (TRIM) protein family involved in various cellular functions such as cell proliferation and differentiation. Bioinformatic analysis, ChIP and luciferase assays revealed that TRIM8 regulation is modulated by four p53 responsive elements located in the first intron of TRIM8 gene. Importantly, we showed that TRIM8 interacts with and increases the p53 protein stability and it affects p53 transcriptional activation of p21 in mammalian cell lines.

TRIM8 is located within the 10q24.3, a region mostly involved in deletions and rearrangements in brain cancer. By QPCR we showed that the relative expression of TRIM8 is strongly underexpressed in a number of human glioblastomas. MTT cell proliferation and colony formation assays showed that the overexpression of TRIM8 inhibits cell proliferation. Consistently, siRNA mediated TRIM8 silencing in mouse neural progenitor cell cultures increases the normal cell cycle progression suggesting that TRIM8 might be involved in a tumour suppression mechanism.

Together these observations suggest the existence of a new p53-TRIM8 feedback-loop mechanism and support the hypotheses that TRIM8 might participate to the development of glioblastomas through yet unknown molecular mechanisms that involve p53.

#### C06.4

##### Identification of Low Penetrance Genes associated to thyroid cancer susceptibility using a two-step case-control approach

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Papillary Thyroid Carcinoma (PTC) is believed to have a strong genetic component, as suggested by the high relative risk (8.6-fold) reported for first-degree relatives of probands. However, no high penetrance gene related to PTC has been described so far. The aim of this study was to identify low penetrance genes (LPG) that could explain the individual susceptibility.

We have selected candidate genes according to the following criteria: (i) their relevant role in biological pathways related to thyroid cell differentiation and proliferation and (ii) their differential expression profile, as observed in our own set of representative samples of thyroid carcinoma. Through this process, 97 genes, tagged by 768 SNPs, were included. SNPs were chosen through different SNPs databases (NCBI, HapMap) and *in silico* tools (Pupa Suite) to include Tag SNPs and putative functional SNPs when possible. These SNPs were genotyped in a two step case-control study. In a first stage, we genotyped more than 600 PTC Spanish patients versus more than 500 representative healthy controls, using the Illumina Sentrix Array platform. Associa-

tion tests were performed on single SNPs and haplotypes to define susceptibility PTC loci. Top ten SNPs are currently being validated by KASPar probes in a second stage of the study that includes an Italian series of more than 400 patients and over 500 controls.

At this moment, we have identified, at least three putative LPG related to thyroid cancer susceptibility. Functional validation is also being performed for two of these loci.

#### C06.5

##### Detection of tumor-specific somatic mutations by transcriptome sequencing of a cytogenetically normal acute myeloid leukemia

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Approximately half of acute myeloid leukemia (AML) patients have at least one chromosomal aberration, whereas the other half classifies as cytogenetically normal (CN-AML). Most of the genetic events that initiate the disease are still undiscovered.

To identify tumor-specific somatic coding mutations, we sequenced the transcriptome of a CN-AML and a matched remission sample by second-generation sequencing technology (Illumina GAI). SNPs were called with the MAQ software. Additional filters were applied to exclude known and possible sequencing artefacts.

We generated 20.4 and 15.6 million 32 bp paired-end reads of the CN-AML and remission sample, respectively, which mapped to exons of UCSC genes. 8.9% of reads for the AML and 5.0% reads of the remission sample mapped to intergenic regions. Of the 11178 transcripts with a higher expression than 60 reads per gene (corresponding to approximately 1 transcript per cell), we sequenced 5911 with an average coverage of greater than seven. By comparing the 63159 SNPs discovered in the CN-AML sample with the respective results in the remission sample, we identified 5 non-synonymous mutations not present in either the remission sample or in dbSNP. Among them is a nonsense mutation affecting the RUNX1 gene, which forms a well known fusion gene in AML (RUNX1/RUNX1T1). The other 4 mutations were missense mutations which need further confirmation. Two of these were in tumor-associated genes (TLE4, FOSB). These results demonstrate that our technique of transcriptome sequencing is an efficient method to discover new mutations in AML.

#### C06.6

##### Ikaros is a frequently affected hematopoietic differentiation factor in pediatric relapse-prone precursor B-cell acute lymphoblastic leukemia

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Relapse is the most common cause of treatment failure in childhood acute lymphoblastic leukemia (ALL), but is difficult to predict in the majority of cases. To explore the prognostic impact of recurrent genomic abnormalities on relapse in children diagnosed with precursor-B cell ALL, we have performed genome-wide copy number profiling of 34 paired diagnosis-relapse samples. Results were validated using locus-specific copy number screening in 200 diagnosis samples of children with or without relapse.

The majority of the copy number abnormalities were preserved between matched diagnosis and relapse samples, but lesions unique in either of the two samples were observed in 82% of the cases. In 68% of the cases, lesions present at diagnosis were no longer detected in relapse samples indicating that these lesions were secondary events, absent in the original therapy-resistant progenitor clone. However, lesions in *IKZF1*, which encodes the hematopoietic differentiation factor Ikaros, were always preserved in relapse. Sequence analysis revealed

that point mutations in *IKZF1* do occur but are less frequent. Validation in a large cohort showed that deletions of exons 3-6, encoding the DNA-binding Zn-finger domains, were most common. Furthermore, *IKZF1* deletions were clearly enriched in leukemias that relapsed (26%) compared to non-relapse cases (11%,  $P=0.006$ ).

In conclusion, *IKZF1* deletions are frequent events in therapy-resistant clones of relapse-prone pediatric precursor B-ALL. Screening for *IKZF1* lesions may help to predict disease outcome.

#### C07.1

##### **Mutations of the SYCP3 gene in women with recurrent pregnancy loss**

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Aneuploidy, a chromosomal numerical abnormality in the conceptus or fetus, occurs in at least 5% of all pregnancies and is the leading cause of early pregnancy loss in humans. Accumulating evidence suggests that the correct segregation of chromosomes is achieved by events occurring in prophase during meiosis I. These include a synapsis between homologous chromosomes, cohesion between sister chromosomes, and meiotic recombination. In our current study, we demonstrate that mutations in SYCP3, a gene encoding an essential component of the synaptonemal complex that is central to the interaction of homologous chromosomes, contributes to recurrent pregnancy loss. Two out of 26 women with recurrent pregnancy loss of unknown cause were found to carry independent heterozygous nucleotide alterations in this gene, neither of which was present among a group of 150 control fertile women. Analysis of transcripts from mini-genes harboring each of these two mutations revealed that both affected normal splicing possibly resulting in the production of a C-terminally mutated proteins. The mutant proteins were found to interact with their wild-type counterpart in vitro and inhibit the normal fiber formation of the SYCP3 protein when co-expressed in a heterologous system. These data suggest that these mutations are likely to generate an aberrant synaptonemal complex in a dominant-negative manner and contribute to abnormal chromosomal behavior that may lead to recurrent miscarriage. Combined with the fact that similar mutations have been previously identified in two males with azoospermia, our current data suggest that sexual dimorphism in response to meiotic disruption occurs even in humans.

#### C07.2

##### **Copy number changes in patients with disorders of sex development**

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Disorders of sex development (DSD), ranging in severity from genital abnormalities to complete sex reversal, are surprisingly common and as such represent a major pediatric concern. The cause of these disorders is most often a disruption of the genetic programs that regulate development of testes or ovaries. Although a number of genes have been identified in these developmental pathways, in many cases of DSD the causative mutations cannot be identified.

We have used the Affymetrix 6.0 whole genome SNP array to perform whole genome copy number analysis on genomic DNA from 33 individuals with gonadal dysgenesis. Rearrangements affecting known sex determination genes were a duplication of the X chromosome including DAX1 in a 46,XY female, as well as a 1.2 Mb deletion upstream of the SOX9 locus that caused sex-reversal without campomelic dysplasia in a 46,XY female. Several other potentially causative rearrangements were identified, including a duplication of the SOX3 gene in a 46,XX male and a 50 kb deletion immediately downstream of GATA4 in a 46,XY female. Both of these genes have been suggested to play a role in gonadal development in animal models without previous supporting evidence in humans.

These findings will stimulate molecular analysis of a number of genes in gonadal determination and differentiation, and support the hypothesis that mutations affecting the regulation of known genes are responsible for a significant number of DSD cases.

#### C07.3

##### **Afamin deficiency in mice leads to reversible infertility**

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Previous work from our group has established the vitamin E-binding property of afamin, a member of the albumin gene family. Afamin is expressed primarily in liver and kidney and secreted into the plasma. Significant amounts were detected also in follicle and seminal fluid suggesting possible roles for afamin in vitamin E transport in these body fluids with potential significance for fertility.

In order to investigate the physiological role of afamin in detail, afamin-overexpressing transgenic and gene-knock-out mice were created and characterised. Transgenic animals were phenotypically without pathological findings. A detailed histological analysis of their testes revealed, however, a significantly higher density of testis tubules. Chimeric (partial afamin-knockout) mice had undetectable afamin blood levels and were completely infertile so that homozygous afamin-knockout mice could not be bred. Histological characterisation of male chimeric animals indicated impaired/dysfunctional spermiogenesis resembling the human Sertoli-Cell-Only phenotype, female animals were histologically free of pathological findings. Supplementation with recombinantly produced murine afamin by a constant diffusion pump device led to restoration of normal testes size, histology, spermiogenesis and fertility.

Taken together, afamin is described here as a novel gene which plays a key role in fertility. The findings from successfully treating infertile mice with exogenous afamin suggest a therapeutic potential for treating human fertility with afamin.

#### C07.4

##### **The challenge of prenatal and preimplantation genetic diagnosis of mitochondrial DNA disorders**

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Mitochondrial DNA (mtDNA) mutations cause a wide range of serious genetic diseases with high transmission risk, due to their maternal inheritance. Because there is no efficient therapy for these disorders, "at risk" couples often ask for prenatal (PND) and/or preimplantation diagnosis (PGD). However, little is known about the factors that might determine the mutant loads (heteroplasmy) in a child of a carrier mother. Here we report our experience in PND and PGD for the 2 most common mtDNA mutations. The m.3243A>G, a cause of MELAS syndrome (Mitochondrial myopathy, Encephalopathy, Lactic Acidosis and Stroke-like episodes), and the m.8993T>G responsible for NARP (Neurogenic weakness, Ataxia, Retinitis Pigmentosa) or Leigh syndrome, were quantified in 21 preimplantation embryos (3 NARP and 18 MELAS) and 19 fetuses (9 MELAS et 10 NARP) from heteroplasmic females. While the mutant load pattern depended on the type of mtDNA mutation, it was however stable in various blastomeres from a given embryo and in various tissues from a given fetus. No temporal variation of heteroplasmy was found in 8 heteroplasmic fetuses who had at least two samples at different stages of development.

Eight children, carrying less than 30% mutant load in the prenatal period, were born and are healthy at 18 months to 9 years of age. Prenatal heteroplasmy less than 30% is therefore predictive of a good postnatal prognosis, even though longer clinical follow-up is required. PGD, by giving the opportunity to select embryos with low mutant loads, constitutes a valuable alternative to PND.

**C07.5****Prenatal oligo-based arrayCGH with custom made focused design: first experiences**

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ArrayCGH is a well implemented diagnostic tool for the detection of submicroscopic chromosomal imbalances. Postnatal array-CGH is routinely performed in our lab using whole genome oligonucleotide arrays (Agilent). Sometimes, if imbalances with uncertain clinical significance are detected, the interpretation of the results is challenging. This is particularly crucial in a prenatal setting. To minimize such difficulties, we decided to design our own focused oligonucleotide microarray using Agilents eArray tool. Our array design is based on the 44k format (more than 40.000 oligonucleotides) and restricted on the dense coverage of about 130 microdeletion/duplication syndromes and subtelomeric regions combined with a 1Mb whole genome spacing.

In our ongoing process of validation we found very good concordance of the arrayCGH results with our focused design compared to the whole genome design. First we used DNA samples derived from blood of patients with known aberrations and normal male and female controls. But the interest in prenatal testing of fetuses with abnormal ultrasound findings is increasing and therefore we successfully tested the DNA from fetal samples like chorion villi and cultured amniocytes. If there is no time for culturing, uncultured amniocytes is a challenging material for use in arrayCGH, since the number of cells in amniotic fluid in early pregnancies is low. Different methods for DNA isolation, whole genome amplification and fluorescence labelling were tested to optimize arrayCGH quality.

Summarized, our results are encouraging to go on with implementing this focused array design in our lab for pre- and postnatal arrayCGH diagnostics.

**C07.6****Trend analysis of invasive prenatal diagnosis before and after the introduction of a new prenatal screening policy in the Netherlands.**K. D. Lichtenbelt<sup>1</sup>, B. Z. Alizadeh<sup>2</sup>, P. G. Scheffer<sup>3</sup>, G. C. Page-Christiaens<sup>3</sup>, P. Stoutenbeek<sup>3</sup>, G. H. Schuring-Blom<sup>4</sup>;

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In 2007 a new prenatal screening-policy for Down's syndrome was introduced in the Netherlands. Before 2007 only women of 36 years and older were offered prenatal screening. According to new legislation, this was extended to all women, regardless of maternal age. Screening includes maternal blood markers and fetal nuchal translucency measurement. We sought to study the effect of the new policy on the outcome of invasive prenatal diagnosis by chorionic villus sampling and amniocentesis. Therefore we collected the outcome of conventional karyotyping of all invasive procedures (n=9931) performed between January 2000 and December 2008 in the University Medical Center Utrecht (UMCU). Data were extracted from the cytogenetic database. A trend analysis was made of the number- and type of abnormal karyotypes. Results show that the contribution of women younger than 36 years rose significantly, from 19,3 % in the years before the new policy, to 25,3% after 2007. For women younger than 36 years, the percentage of abnormal karyotypes also increased significantly from 13% to 19%. The percentage of abnormal karyotypes for all maternal ages increased from 6,5% to 8,8%, mainly due to the increased use of high performing indications, such as 'abnormal first trimester screening' and 'sonography findings', as opposed to 'advanced maternal age' alone as indication for the invasive diagnostics. This rise was mainly due to the increased detection of autosomal trisomies. There was no significant difference in the type of autosomal trisomies detected.

**C08.1****Nicolaides-Baraitser Syndrome - Delineation of the Phenotype**S. B. Sousa<sup>1,2</sup>, O. A. Abdul-Rahman<sup>3</sup>, A. Bottani<sup>4</sup>, V. Cormier-Daire<sup>5</sup>, A. Fryer<sup>6</sup>,G. Gillessen-Kaesbach<sup>7</sup>, D. Horn<sup>8</sup>, D. Josifova<sup>9</sup>, A. Kuechler<sup>10</sup>, M. Lees<sup>1</sup>, K.

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Nicolaides-Baraitser syndrome is a mental retardation-multiple congenital anomalies syndrome, reported for the first time in 1993, and since then in only four cases. We aimed to delineate the phenotype and natural history better and were able to gather eighteen hitherto undescribed patients through a multi-centric collaborative study. In addition, we gathered follow-up data of the earlier reported cases, including long-term follow-up of the original patient.

Nicolaides-Baraitser syndrome was found to be a distinct and well recognizable entity with limited clinical variability. Main clinical features are severe mental retardation, limited or absent speech, seizures, short stature, sparse hair, typical facial characteristics, brachydactyly, prominent finger joints and broad distal phalanges. Some of the features can be progressive with time. There is no important gender difference in occurrence, frequency and severity of the syndrome, and all cases have thus far been sporadic. Consanguinity is not increased. Micro-array analysis in 14 of the patients gave normal results. There is no clue to the cause, except possibly the progressive nature.

The entity has always been considered a very rare condition, but the present series gathered over a short period of time may indicate it to be underrecognized. The present detailed phenotype analysis may help recognizing further patients. Further research to detect the cause is in progress.

**C08.2****Severe Non-Lethal Recessive Type VIII OI: Clinical, Histological and Radiographic Features**J. C. Marin<sup>1</sup>, W. Chang<sup>1</sup>, F. H. Glorieux<sup>2</sup>, T. E. Hefferan<sup>3</sup>, F. Rauch<sup>2</sup>, M. Abukhaled<sup>1</sup>, P. A. Smith<sup>4</sup>, D. Eyre<sup>5</sup>,

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Type VIII osteogenesis imperfecta is a recently defined recessive lethal/severe OI caused by null mutations in LEPRE1 encoding collagen prolyl 3-hydroxylase I. We present here the first complete description of two non-lethal cases of type VIII OI, 17 and 10 yr old boys with null mutations in both alleles of LEPRE1. Both probands were SGA term babies. They have extreme growth deficiency, white sclerae and normal dentin. Their extremities are rhizomelic with popcorn epiphyses. They have severe scoliosis with multiple vertebral compressions; DEXA L1-L4 z-scores were -6.3 and -5.8. Their dermal collagen fibrils have same average diameter as matched controls, but greater diameter variability and multiple border irregularities. On mass spectrometry, the level of Type I collagen Pro986 3-hydroxylation was <5% of normal in dermis, iliac crest bone and collagen secreted by cultured fibroblasts and osteoblasts, ruling out redundancy of P3H function in skin and bone. Serum test results were distinctive compared to other OI types,

with elevations of both BSAP, an osteoblast product, and TRAP, an osteoclast product, consistent with elevated bone turnover. Iliac crest histomorphometry confirmed extremely high bone turnover, along with elevated mineral apposition rate and faster matrix mineralization than type VII OI. Stained sections demonstrated a distinctive broad osteoid seam on all trabecular surfaces and abnormal osteoblast morphology, with irregularly shaped cells piled up on the newly deposited matrix, rather than a normal monolayer of cuboidal osteoblasts. These clinical and histological features provide a diagnostic guide for clinicians.

#### C08.3

#### Aicardi-Goutières syndrome and other disorders associated with intracranial calcification

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Aicardi-Goutières syndrome is a Mendelian mimic of congenital infection, also showing overlap with systemic lupus erythematosus at both a clinical and biochemical level. The recent identification of mutations in TREX1 and genes encoding the RNASEH2 complex in AGS patients, and studies of the function of TREX1 in DNA metabolism, have defined a novel mechanism for the initiation of autoimmunity by interferon-stimulatory nucleic acid. In a genotype-phenotype analysis, we previously showed that 17% of AGS families do not have identifiable mutations in AGS1-4. We have now identified the AGS5 gene and will present data to show that the AGS5 protein may (also) act as a negative regulator of the cell-intrinsic antiviral response.

Additionally, through our work on Aicardi-Goutières syndrome we have developed an interest in several 'new' syndromes where intracranial calcification provides a significant clue to the diagnosis. These disorders include Coats plus/cerebroretinal microangiopathy with calcification and cysts (CRMCC), spondyloenchondroplasia with CNS disease and immune dysfunction (SPENCD), and (what we have called) 'true-pseudo-TORCH syndrome'. We will outline our ongoing clinical and molecular efforts to classify diseases in which intracranial calcification provides an important diagnostic handle.

#### C08.4

#### Gerodermia osteodysplastica is caused by mutations in SCYL1BP1, a novel Rab-6 interacting golgin

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Gerodermia osteodysplastica (GO; OMIM 231070) is a rare autosomal recessive segmental progeroid syndrome characterized by osteoporosis with increased fracture susceptibility, joint laxity with frequent hip dislocation, cutis laxa and jaw hypoplasia. Using a positional cloning approach in consanguineous Mennonite pedigrees from Germany, Canada, and Mexico, we identified an identical homozygous interval on chromosome 1q24 with a multipoint lod score of 12.0. Subsequent mutation screening revealed the homozygous nonsense mutation p.Glu143X in the gene SCYL1BP1. In nine additional GO patients from various origins we identified eight other loss-of-function mutations. SCYL1BP1 encodes the soluble protein SCY1-like 1 binding protein 1, which is expressed at high levels in skin and osteoblasts and contains coiled-coil domains. Protein expression was completely lost in patient fibroblasts. We demonstrated that SCYL1BP1 localizes to the Golgi apparatus and specifically interacts with GTP-bound small GTPase Rab6, an important regulator of anterograde and retrograde Golgi trafficking.

In contrast to the overlapping disorder Debré-type cutis laxa (ARCL type II, OMIM 278250) no impairment of retrograde trafficking was detected in patient fibroblasts. Therefore, it is likely that the novel golgin SCYL1BP1 plays a role in anterograde trafficking within the secretory pathway. These findings imply an association of secretory pathway dysfunction with age-related changes in connective tissues.

#### C08.5

#### Familial cases with hypomethylation of the imprinted IGF2-H19 domain in Silver-Russell Syndrome (SRS)

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Silver-Russell syndrome (SRS) is a heterogeneous condition characterized by severe intrauterine growth retardation, poor postnatal catch up growth, craniofacial features, body asymmetry and a variety of minor malformations. Loss of DNA methylation at the telomeric imprinting control region 1 (ICR1) on 11p15 is an important cause of SRS.

We studied the methylation pattern at the H19-IGF2 locus in 200 patients with SRS and SRS-like phenotypes and identified epimutations in about 40% of patients with SRS. Amongst this cohort we identified two families each with two siblings with SRS, displaying hypomethylation of H19 and IGF2. In both families neither of the parents showed clinical signs of SRS and methylation analysis in the fathers revealed normal results. In a third family we identified an epimutation in both a 30 year-old clinically affected father and his likewise affected daughter. Father and daughter showed a classical SRS phenotype with no additional clinical signs. Sequencing of the differentially methylated region (DMR) 5' of the H19 gene did not reveal mutations in the three families.

The two families for whom we identified epimutations in siblings most likely represent germ cell mosaicism of an incorrect methylation mark at the ICR1 during spermatogenesis in the fathers. The third family accounts for transmission of an epimutation from an affected father to his daughter through the male germ line. The underlying mechanism remains to be explained.

Our finding of familiar cases with SRS carrying epimutations has important implications for both genetic counseling and determination of the origin of the epimutations.

#### C08.6

#### Germline mutation in NLRP2 (NALP2) in a familial imprinting disorder (Beckwith-Wiedemann Syndrome).

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Beckwith-Wiedemann syndrome (BWS) is a fetal overgrowth and human imprinting disorder resulting from the deregulation of a number of genes, including IGF2 and CDKN1C, in the imprinted gene cluster on chromosome 11p15.5. Most cases are sporadic and result from epimutations at either of the two 11p15.5 imprinting centres (IC1 and IC2). However, rare familial cases may be associated with germline 11p15.5 deletions causing abnormal imprinting *in cis*. We report a family with BWS and an IC2 epimutation in which affected siblings had inherited different parental 11p15.5 alleles excluding an *in cis* mechanism. Using a positional-candidate gene approach we found that the mother was homozygous for a frameshift mutation in exon 6 of NLRP2. While germline mutations in NLRP7 have previously been associated

with familial hydatidiform mole, this is the first description of *NLRP2* mutation in human disease and the first report of a *trans* mechanism for disordered imprinting in BWS. These observations are consistent with the hypothesis that *NLRP2* has a previously unrecognised role in establishing or maintaining genomic imprinting in humans.

#### C09.1

##### Genetic risk model for coeliac disease helps identify high-risk individuals.

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**Background:** Coeliac disease (CD) is a common chronic disorder of the small intestine, resulting from aberrant cellular responses to gluten peptides, and often remains undiagnosed. It is a complex genetic disorder although 95% of the patients carry the risk heterodimer HLA-DQ2. Genome-wide association studies on CD have identified nine non-HLA loci that also contribute to CD risk, most of which are shared with other immune-related diseases. Our aim is to predict the genetic risk for CD using HLA and non-HLA risk alleles.

**Methods:** We selected ten independent polymorphisms in 2308 cases and 4585 controls from Dutch, UK, and Irish populations and categorized the individuals into three risk groups, based on their HLA-DQ2 genotype. We used the summed number of non-HLA risk alleles per individual to analyze their cumulative effect on CD risk, adjusting for sex and population group in logistic regression analysis. We validated our findings in 436 Italian cases and 532 controls.

**Findings:** CD cases carried more non-HLA risk alleles than controls: individuals carrying 13 or more risk alleles had a higher CD risk (OR = 6.2; 95% CI 4.1-9.3) compared to those carrying zero to five risk alleles. Combining HLA and non-HLA risk genotypes in one model increases sensitivity by 6.2% compared to using only HLA for identification of high-risk individuals with slight decrease in specificity.

We can use non-HLA risk factors for CD to improve identification of high-risk individuals. Our risk model is a first step towards better diagnosis and prognosis in high-risk families and population-based screening.

#### C09.2

##### Identity by descent within and between human populations

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The availability of cost-effective, high throughput technologies to genotype common alleles has yielded an unprecedented wealth of genome-wide data on human variation, deeply sampled within and across populations. We have developed a rapid method that facilitates extensive evaluation of shared genetic segments across millions of sample-pairs. We demonstrate the use of hidden relatives as parent-surrogates for phasing detection of deletions, and imputation at IBD segments.

When combined with phenotypic data, IBD provides a strategy to detect association to rare, untyped alleles. This is especially useful in isolated populations. We demonstrate this by detection of previously unreported, genomewide significant associations in Pacific Islanders and Ashkenazi Jewish samples.

In Population genetics, IBD sharing paves the way for observing very recent genetic history of samples, both genomewide as well as for specific loci. We show extensive hidden relatedness between individuals within populations that provides estimates of demographic parameters. Specifically, for Ashkenazi Jewish populations we demonstrate and a severe bottleneck 20-25 generations before present. We show genetic sharing to be focused at regions that suggest a causal mechanism for ancient sharing rather than recent relatedness, such as the HLA and the commonly polymorphic inversion of 5Mbp on chromosome 8p23.1. Finally, we filter out sharing that is non-informative because it is too recent or causal and show clustering of populations based on genetic sharing.

#### C09.3

##### Control of meiotic recombination in the human genome

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Meiotic recombination increases haplotype diversity and thus profoundly affects evolution. High resolution analysis of *de novo* recombination events in human sperm DNA has revealed clustering into very narrow hotspots that generally coincide with abrupt breakdown of linkage disequilibrium. Using population genetics approaches applied to whole genome diversity surveys such as the International HapMap project, sites of historical recombination activity have been inferred. Around 40% of ~30000 hotspots so identified contain a 13 bp motif CCNCCNTNNCCNC which appears to be involved in hotspot specification and which may also drive some modes of genome instability. To test the effect of the motif on crossover frequencies and distributions, candidate hotspots with the motif at their centre and which carry a motif-disrupting SNP are being studied. The first hotspot to be analysed in sperm showed extreme biased gene conversion accompanying crossover in men heterozygous at the disrupting SNP. The bias was in favour of the motif-disrupting allele, consistent with suppression of crossover initiation on chromosomes carrying this allele. This provides direct evidence for the importance of this motif in crossover initiation, and helps shed light on hotspot polymorphism and turnover in human populations.

#### C09.4

##### New evidences about MHC-based patterns of mate choice

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Major Histocompatibility Complex (MHC) genes code for cell surface proteins, which plays an important role in immune recognition. In the late 1970s, Yamazaki observed that inbred mice were more likely to mate with partners having MHC dissimilar genes. Females' preference for MHC dissimilar mates was also observed in other vertebrate species, including humans. It has been shown that MHC influences mating selection mediated by preferences based on body odor. What's the functional significance of these findings, if some? It was assumed that through olfactory cues MHC-related evolved as a strategy to maximize the offspring MHC heterozygosity. Parents with dissimilar MHCs could provide their offspring with a better chance to ward infections off because their immune system genes are more diverse. MHC genotype might be used to signal relatedness and immune response genotypes through.

We investigated whether husband-wife couples (n=90) obtained from LIGH's database were more MHC-similar/dissimilar in comparison to random couples generated from the same database (n=55 000) as to collect evidence of MHC influence in MHC-based patterns of mate choice.

The individuals HLA typing (Class I and Class II) was performed by PCR-SSP or PCR-rSSOP using a commercial kit ( One Lambda Inc., Canoga Park. CA, USA).

Our results and comparisons ( p= 0,014) suggest that couples seem to be formed by individuals with less HLA similarity, corroborating the hypothesis that HLA antigens, especially Class I, may influence mate selection and marriages in humans.

#### C09.5

##### Genomic tests for identification of authenticity of historical remains in case of Romanov family.

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We performed the DNA investigation of recently found human historical remains presumably belonging to Prince Alexei and his sister, children of Russian Emperor Nicholas II. We also provided the comprehensive genomic analysis of authenticity of skeleton remains of Nicholas II and

members of his family and their attendants. The rapid methodological approach for reconstruction of complete mitochondrial DNA sequences from very limited amount of old historical human specimens has been developed. We were able to recover the highly informative nuclear DNA: gender-, autosomal STRs and Y-STR profiles from the >90 years old bone specimens or the archival bloodstain samples. Multiplex PCR assay and population databases analysis were performed to test the origin of Y-chromosome haplogroups of Nicholas II and Prince Alexei. Mitochondrial haplogroups were also defined for all members of Romanov family and their attendants. Comparison with large population databases from Eurasian populations was employed to test whether the genographic origin of the haplotypes is consistent with the historical data.

We demonstrate here that convergent analysis of complete mitochondrial genome sequences combined with Y-chromosome profiles is efficient for individual and kinship identification of historical relics. The genotyping of damaged specimens and paternal and maternal lineages of Royal family demonstrated that recently found 90-years old human remains belong to Nicholas II children, Prince Alexei and his sister, and provided an evidence that remains of all members of Romanov family (including Anastasia and Alexei) have been identified.

#### C09.6

#### Dissecting the genetic make-up of Central Eastern Sardinia using a high density set of sex and autosomal markers

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Genetic isolates are valuable for identifying genetic variations underlying complex traits. However, prior knowledge of the genetic structure of the isolate is fundamental for carrying-out genome-wide association studies (GWAS) in these populations. The Sardinian population is currently the target of GWAS because of its ancient origin and long-standing isolation. To perform GWAS in Sardinia, we aim to characterize a subpopulation from the archaic area of Central-Eastern Sardinia at the genomic level. We used sex-specific markers (Y-chromosome and mtDNA) to assess the heterogeneity of the founder lineages and the divergence from other populations. In addition, we used a dense set of autosomal markers (SNP 5.0 array, Affymetrix) to investigate genome-wide Linkage Disequilibrium, to construct a Copy Number Variation map and to estimate pair-wise kinship and inbreeding. We first determined Y-chromosome lineages in 256 unrelated Sardinians using biallelic and microsatellite markers. Our analysis showed that the frequency of the major Y haplogroups clearly sets this population apart from other European haplogroups. The analysis of microsatellite markers revealed a high degree of gene diversity. Pairwise kinship and inbreeding were estimated in 113 subjects using 77709 autosomal SNP markers. We found that 16% of the subject pairs shared identical-by-descent alleles more often than expected by chance. Furthermore, 60% of the subjects had low inbreeding coefficient values. Our preliminary results confirm that Sardinia is genetically different from other populations, as shown by Y-chromosome markers. The kinship and inbreeding estimates indicate some degree of relatedness among Sardinians, as expected for an isolated population.

#### C10.1

#### A genome-wide association study identified novel susceptibility loci for type 2 diabetes mellitus in Chinese population residing in Taiwan

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Type 2 diabetes mellitus (T2DM) is the forth leading cause of death in Taiwan. The prevalence of DM in Taiwan increases from 4.63% in 2001 to 4.69% in 2003 and the mortality from diabetes mellitus has almost doubled over the past ten years. To identify genetic variants for T2DM in Han-Chinese that accounts for 98% of the Taiwan population, we conducted a two-stage genome-wide association study with a total of

1715 cases and 2000 random controls. Genotyping was started with 517,401 SNPs that pass quality control filters using the Illumina Hap550duov3 chip and then validated and replicated in a cross-platform sequenom. All patients were diagnosed using the American Diabetic Association Criteri and recruited from the China Medical University Hospital, Taiwan. Random controls were selected from the Taiwan Han-Chinese Blood and Cell Bank, Academia Sinica, Taiwan. We excluded SNPs from further analyses by three major criterions: (1) missing data rate >5%, (2) missing data rate >1% for SNPs with a minor allele frequency < 5%, and (3) p-value of Hardy-Weinberg Disequilibrium test < 10<sup>-7</sup>. We identified one previously unknown signal with significant evidence (p < 10<sup>-7</sup>) for association with T2DM. Four SNPs/loci showed positive association (10<sup>-7</sup> < p < 10<sup>-5</sup>), including the KCNQ1 previously reported in Japanese population. In addition to confirming the known association with KCNQ1, we have identified four novel loci associated with T2DM in Han-Chinese population. Our study indicated the heterogeneity of type 2 diabetes mellitus between the Asian and Caucasian populations.

#### C10.2

#### Genome-wide association analysis and expression analysis from adipose tissue reveals coagulation factor XIII as a novel candidate gene for low HDL-cholesterol

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Inverse correlation between HDL-cholesterol level and atherosclerosis has been established. To identify potentially novel variants associated with HDL-cholesterol, we genotyped 450 Finns (Illumina370K) from EUFAM population sample, either having extremely high (>90th percentile) or low (<10th percentile) HDL-C levels. Out of these, subcutaneous fat biopsies were obtained from 54 individuals, and their global expression profiles were analyzed using Affymetrix microarrays.

Two best HDL associating SNPs were located at CETP, as in previous studies. However, the 3rd and 10th best hits (rs7766109, p=10<sup>-5</sup> and rs4959377, p=3.45x10<sup>-5</sup>) were located within F13A1, a coagulation factor with no previously reported associations with lipids. After testing 54 additional SNPs within F13A1 region, we detected associations for 10 of them, all of them located within introns 3-5. The associations of F13A1 introns 3-5 for HDL were replicated in a normally distributed Health2000 population sample (n=890). At mRNA level, F13A1 was higher expressed in adipose tissue of individuals with low HDL (p=0.007), and a dose-dependent effect of rs7766109 genotype on both F13A1 expression (p=0.02) and HDL-levels (p=0.004) was observed.

We also analyzed the adipose tissue expression profiles of 5 insulin sensitive and 5 insulin resistant Finnish subjects before and after a euglycemic insulin clamp. Again, F13A1 expression was 6-fold higher in IR subjects compared to IS individuals (p=0.003). Moreover, an insulin-induced decrease in F13A1 expression was observed in IR (p=0.047) but not IS subjects.

Here we show that combination of global SNP and expression analysis can be a powerful tool in studying the mechanisms of complex diseases.

#### C10.3

#### Loci on chromosome 19 and 20 are associated with age at natural menopause: a meta-analysis of 10,399 women

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Menopause, defined by the cessation of the menstrual cycle due to depletion of the follicle pool, influences a woman's well-being and is an important risk factor for several major age-related diseases including cardiovascular diseases, osteoarthritis and osteoporosis. The heritability of age at natural menopause is estimated to be ~60%, suggesting a strong genetic component. While few candidate gene polymorphisms are associated with age at menopause, the genetic risk factors are largely unknown.

We conducted a meta-analysis of genome-wide association studies with >2,500,000 SNPs for age at natural menopause in 10,399 post-menopausal Caucasian women from 9 population-based cohorts. We identified 20 SNPs on chromosome 19q13.4 and chromosome 20p12.3 that reached genome-wide significance (p-value < 5\*10<sup>-8</sup>). The chromosome 19 SNPs are located on the same locus in BRSK1 and LOC284417, the most significant SNP is associated with 0.47 year earlier menopause per allele copy (SE: 0.05, p-value 1.9\*10<sup>-18</sup>). BRSK1 is a brain-specific serine-threonine kinase, for which no link with menopause could be established. The top hit on chromosome 20 is located in MCM8, and is associated with an 0.89 year earlier menopause per copy of the minor allele (SE: 0.11, p-value: 2.2\*10<sup>-15</sup>). MCM8 is expressed in mouse ovaries and is involved in DNA replication. No other loci reached genome-wide significance.

Our results provide evidence for common genetic variants regulating the timing of ovarian aging although the precise mechanisms are unknown. Additional studies are warranted to identify the causal variants at these loci and to characterize their functional significance.

#### C10.4

##### Genome-wide association scan for bilirubin levels in a Sardinian population

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Bilirubin is the final product of heme degradation. Unconjugated bilirubin is transported into hepatocytes, where it is glucuronidated by UGT1A1 and secreted into the bile canaliculi in its conjugated form. Recent studies have shown that elevated bilirubin levels in adults are inversely associated with the risk of developing coronary artery disease and directly with gallstones, while unconjugated hyperbilirubinemia may be associated with susceptibility to drug toxicity. The genetic factors so far identified cannot fully explain the trait heritability, estimated to be around 45%. To identify additional loci, we conducted a genome-wide association scan (GWAS) in 4300 Sardinians from the SardiNIA project, genotyped using the Affymetrix 500K and 10K Arrays, and evaluated the additive effect of 362,129 SNPs that passed quality control tests. The GWAS results revealed, in addition to two known loci,

UGT1A1 (p=6.2x10<sup>-62</sup>) and G6PD (p=2.5x10<sup>-8</sup>), a strong association on chromosome 12p12.2 (p=3.9x10<sup>-9</sup>). The findings were replicated in an independent sample of 1860 Sardinians and in 832 Amish individuals from the HAPI Heart Study (overall pvalue<5x10<sup>-14</sup>). Interestingly, we observed in all three cohorts that the association with unconjugated bilirubin was stronger than with conjugated bilirubin. Finally, we found an enrichment of the high bilirubin levels allele at 12p12.2 in patients with idiopathic mild unconjugated hyperbilirubinemia lacking mutations in the UGT1A1 gene, suggesting that this locus may be involved in the regulation of serum bilirubin levels in the general population and in some bilirubin-related disorders that are only partially explained by variants in previously identified loci.

#### C10.5

##### Identification of a Shared Genetic Susceptibility Locus for Coronary Heart Disease and Periodontitis

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Recent studies indicate a mutual epidemiological relationship between coronary heart disease (CHD) and periodontitis. Both diseases are associated with similar risk factors and are characterized by a chronic inflammatory process. In a candidate-gene association study we identify an association of a genetic susceptibility locus shared by both diseases. We confirm the known association of two neighboring linkage disequilibrium regions on human chromosome 9p21.3 with CHD, and show the additional strong association of these loci with the risk of aggressive periodontitis. For the lead SNP of the main associated linkage disequilibrium region, rs1330348, the odds ratio of the autosomal-recessive mode of inheritance is 1.99 (95% confidence interval 1.33-2.94; P = 6.9 x 10<sup>-4</sup>) for generalized aggressive periodontitis, and 1.72 (1.06-2.76; P = 2.6 x 10<sup>-2</sup>) for localized aggressive periodontitis. The two associated linkage disequilibrium regions map to the sequence of the large antisense noncoding RNA ANRIL which partly overlaps regulatory and coding sequences of CDKN2A/CDKN2B. A closely located diabetes associated variant was independent of the CHD and periodontitis risk haplotypes. Our study demonstrates that CHD and periodontitis are genetically related by at least one susceptibility locus, which is possibly involved in ANRIL activity and independent of diabetes associated risk variants within this region. Elucidation of the interplay of ANRIL transcript variants and their involvement in increased susceptibility to the interactive diseases CHD and periodontitis promises new insight into the underlying shared pathogenic mechanisms of these complex common diseases.

#### C10.6

##### Meta-analysis of genome-wide scans identifies three novel loci influencing central obesity including one with a women-specific association with waist-hip-ratio

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Central obesity, which is an abnormal accumulation of intra-abdominal fat, is a major risk factor for cardiovascular disease (CVD) and type 2 diabetes. Measures of central obesity and fat distribution have been shown to be highly heritable. To identify the influencing genetic loci, a meta-analysis of 16 genome-wide scans of 38,580 Caucasians of European-ancestry was performed for waist circumference (WC) and

waist-hip-ratio (WHR), two well-measurable and ubiquitously available surrogate measures for central obesity.

The top associating variants from this first stage were followed by de novo genotyping (N=56,859) and in silico replication (N=13,830) for 27 SNPs with strong association with WC or WHR, but no or only a weak association with BMI or height. Two loci reaching genome-wide significance, one at 6p12 (P for WC = 1.9x10-11), one at 8p23 (P for WC=8.9x10-9) were strengthened by the CHARGE consortium (N=31,373). One further locus at 1q41 (P for WHR = 2.6x10-8) was genome-wide significantly associated in the women-specific analysis. The WHR women-specific locus (1q41) exhibits association even after controlling for body-mass-index, is associated also with percentage body fat from bioimpedance measures, and most intriguingly shows reciprocal effects on triglycerides in-line with the lipase role of a nearby gene. For the 6p12 locus, preferential expression in adipose tissue is reported, but association was also strong with body-mass-index. In summary, the results from these genome-wide meta-analyses including the first scan on WHR suggest novel loci/genes for regulating central obesity and highlight the complementary genetics of waist phenotypes to using body-mass-index.

#### C11.1

#### An aCGH screening study in 150 patients identifies a novel dosage-sensitive gene, TAB2, which is disrupted in multiple patients with cardiac defects

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An array-CGH screening study of 150 patients with congenital heart defects (CHDs) at 1Mb resolution identified 41 imbalances not known to be CNP. Our genetic decision algorithm classified CNVs as causal in 25 patients (17%). Systematic analysis of clinical features of the 150 patients showed dysmorphism as the only parameter significantly associated with detection of a causal CNV at this resolution. To assess if CNVs below 1Mb are involved in syndromic CHDs, we reanalyzed 30 patients on Agilent 244K array: 2 causal CNVs were detected.

Mapping chromosomal aberrations is an established strategy for gene identification. Our array-CGH screening identified a 6q24.2q25.1 deletion in a patient with VSD and coarctation. Genotype-phenotype correlations in 9 additional deletion patients identified a critical 1.2 Mb region on 6q25.1 harboring 11 genes. Prioritization of all candidate genes on 6q24q25 yielded a ranked list, with TAB2 (located in the critical deletion region) ranking first, suggesting TAB2 haplo-insufficiency causes errors in heart development.

To reinforce this conclusion, we demonstrated that TAB2 is expressed in the heart of both human and zebrafish embryos. We moreover titrated a *tab2* knock-down in developing zebrafish and showed that halving *tab2* dosage leads to broad developmental problems including heart defects. Interestingly, *Tab2*<sup>+/−</sup> mice were reported to die perinatally for unknown reasons.

Mutation analysis of TAB2 in 100 patients with a CHD failed to identify pathogenic mutations. However, in a small family where a balanced translocation segregates with CHDs, we demonstrate that TAB2 is disrupted showing that TAB2 haplo-insufficiency causes CHDs in humans.

#### C11.2

#### Shox2 mediates Tbx5 activity by regulating Bmp4 in the sinus venosus of the developing heart

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Heart formation requires a highly balanced network of transcriptional activation of genes. The homeodomain transcription factor Shox2 is essential for the formation of the sinoatrial valves and for the development of the pacemaking system. Here we identify the *Bmp4* gene as the first direct target of Shox2. Shox2 interacts directly with the *Bmp4* promoter and activates transcription in luciferase reporter assays. In addition, ectopic expression of *Shox2* in *Xenopus* embryos stimulates transcription of the *Bmp4* gene and silencing of *Shox2* in cardiomyocytes leads to a reduction in *Bmp4* expression. In *Tbx5*<sup>del/+</sup> and *Shox2*<sup>−/−</sup> mice we show that the T-box transcription factor Tbx5 is required for *Shox2* expression in the inflow tract and that *Bmp4* is regulated by *Shox2* in this compartment of the embryonic heart. This work contributes to the unravelling of the intricate interplay between the heart-specific transcriptional machinery and developmental signalling pathways.

#### C11.3

#### Pharyngeal ectoderm to neural crest signalling is disrupted in a mouse model of DiGeorge syndrome

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**Background/Aims:** Haploinsufficiency of Tbx1 is a major cause of the DiGeorge and velocardiofacial syndromes. It is now apparent that the requirements for Tbx1 vary by tissue and embryonic stage. As part of an effort to unravel the function of Tbx1 we conducted microarray analysis of cells flow sorted from Tbx1 null and heterozygous embryos. A methodological aim was to validate fluorescent β-galactosidase substrates for FACS.

**Method and Materials:** FACS-GAL derived Tbx1 expressing cells from E9.5 day embryos provided RNA for Affymetrix array analysis. Potential target genes were analysed for function and epistasis in both fish and mouse. Tbx1(enhancer) Cre, Ap2Cre, and FoxA2Cre were crossed into conditional alleles to assess tissue specific requirements for one candidate target gene, Gbx2.

**Results and Conclusions:** Gbx2 was downregulated in two separate microarray and RTQPCR experiments. Gbx2 and Tbx1 were in epistasis in both a zebrafish and mouse model of DiGeorge syndrome, strongly suggesting the involvement of the Gbx2 transcription factor in pathways downstream of Tbx1. This epistasis involved the remodelling of the fourth pharyngeal arch artery leading to interruption of aortic arch type B - the cardiovascular defect most specific for the syndrome. We found that, for correct remodelling, Gbx2 was required in the embryonic ectoderm overlying the pharyngeal arches, implying ectodermal signalling downstream of Gbx2. We provide evidence that Slit and Robo expression, in ectoderm and cardiac crest respectively, is disrupted in Tbx1/Gbx2 mutant embryos. For the first time, we demonstrate abnormal neural crest patterning in Tbx1 heterozygous mice.

#### C11.4

#### Positive and negative feedback regulates the transcription factor FOXL2 in response to cell stress: evidence for a regulatory imbalance induced by disease-causing mutations

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FOXL2 is a Forkhead transcription factor, essential for ovarian function, whose mutations are responsible for the Blepharophimosis Ptosis Epicanthus-inversus Syndrome (BPES), characterized by craniofacial defects, often associated with premature ovarian failure. We show that cell stress upregulates FOXL2 expression in an ovarian granulosa cell model. Increased FOXL2 transcription might be mediated at least partly by self-activation. Using 2D-western blot, we show that the response of FOXL2 to stress correlates with a dramatic remodeling of its post-translational modification profile. Upon oxidative stress, we observe an increased recruitment of FOXL2 to several stress-response promoters, notably that of the mitochondrial Superoxide Dismutase (*MnSOD*). Using several luciferase reporter systems, we show that FOXL2 transactivation is markedly enhanced in this context. Models predict that gene upregulation in response to a signal should eventually be counterbalanced to restore the initial steady state. In line

with this, we found that FOXL2 activity was repressed by the SIRT1 deacetylase. Interestingly, we demonstrate that SIRT1 transcription is, in turn, directly upregulated by FOXL2, which closes a negative-feedback loop. The regulatory relationship between FOXL2 and SIRT1 prompted us the test action of nicotinamide, an inhibitor of sirtuins, on FoxL2 expression/activity. According to our expectations, nicotinamide treatment increases FoxL2 transcription. Finally, we show that 11 disease-causing mutations in the ORF of FOXL2 induce aberrant regulation of FOXL2 and/or regulation of the FOXL2 stress-response target gene *MnSOD*. Taken together, our results establish that FOXL2 is an actor of the stress response, and provide new insights into the pathogenic consequences of FOXL2 mutations.

### C11.5

#### Loss-of-function mutation in the dioxygenase-encoding, obesity-associated *FTO* gene causes severe growth retardation and multiple malformations

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We ascertained a large Palestinian Arab consanguineous multiplex family in which nine affected infants presented a previously unreported polymalformation syndrome including pre- and post-natal growth retardation, severe psychomotor delay, brain malformations, cardiac defects, genital anomalies, cleft palate, characteristic facial dysmorphism and premature death. We mapped the disease-causing gene on 16q12 and subsequently identified a homozygous c.947G>A (p.R316Q) variation within the *FTO* gene, a gene associated with human adiposity. This variant co-segregated with the disease, was not found in 730 control alleles and altered a highly conserved residue. *FTO* has been recently recognized as a member of the AlkB related family of non-haem iron- and 2-oxoglutarate (2OG) dependent dioxygenases and shown to localise in the nucleus. Members of this family directly reverse alkylated DNA and RNA damages by oxidative demethylation and the R316 residue play a crucial role by mediating 2OG-coordination. While the mutation does not alter the nuclear localisation of the protein, western blotting experiments showed a significantly reduced amount of *FTO* protein in patient fibroblasts as compared to controls. More importantly, *in vitro* assays demonstrated that the R316Q *FTO* mutant protein had a markedly reduced activity, likely due to the inability of the mutant protein to interact with the 2OG co-substrate. Finally, patient fibroblasts display signs of senescence including growth defects, limited replicative lifespan and altered cell morphology. Our findings demonstrate an unsuspected role for *FTO* protein in human development and provide the first example of a human disorder related to the defect of an AlkB related protein.

### C11.6

#### Microvillus inclusion disease results from loss of myosin Vb function

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Autosomal recessive microvillus inclusion disease (MVID) presents with an intractable diarrhea within the first few weeks of life. A pathognomonic lack of microvilli on the surface of mature enterocytes and an occurrence of intracellular vacuoles lined by microvilli (microvillus inclusions) are seen in biopsies. Recently, we identified mutations in the MYO5B gene, encoding the unconventional type Vb myosin motor protein in nine MVID patients (Müller et al., Nat Genet 40: 1163-5, 2008). We here evaluated further the role of MYO5B mutations and demonstrate loss of function of MYO5B in MVID. Direct sequencing of

the MYO5B exons and all splice-sites was performed in 11 unrelated MVID patients and revealed 15 novel nonsense and missense mutations. 10 patients showed biallelic mutations and in one patient only one mutation was identified. Immunofluorescence, Western blotting and immunoelectron microscopy were applied to analyze the effects of MYO5B-siRNA knock-down in Caco-2 intestinal epithelial cells. Loss of surface microvilli and cytoplasmic vacuoles containing microvilli were induced in CaCo-2 cells following MYO5B knock-down. We show that MYO5B gene mutations are the major cause of microvillus inclusion disease and MYO5B knock-down recapitulates most of the cellular phenotype in vitro. The finding of a majority of truncating mutations and our in vitro study independently show loss of MYO5B function as the cause of MVID. MYO5B may mobilize recycling endosomes and apical proteins necessary for brush border maintenance.

### C12.1

#### DPF3 - a Novel Epigenetic Regulator of Cardiac Muscle Development and Function

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Chromatin remodeling and histone modifications have a high impact on cardiac function and development. Both facilitate access of transcription factors to DNA by promoting the unwinding and destabilization of histone-DNA interactions. We present DPF3, a novel epigenetic key factor, which plays an essential role in cardiac and muscle development. In a genome-wide gene expression study of congenital malformed human hearts we identified DPF3 as significantly up-regulated in hypertrophic right ventricular myocardium of patients with Tetralogy of Fallot.

Taking a systematic approach including methods such as gene expression arrays, morpholino knockdown, chromatin-immunoprecipitation, GST pulldown and tandem-affinity-purification, we elucidated the function of DPF3. DPF3 is associated with the BAF chromatin remodeling complex and binds methylated and acetylated lysine residues of histone 3 and 4. During development Dpf3 is expressed in the heart and somites of mouse, chicken and zebrafish. Morpholino knockdown of *dpf3* in zebrafish leads to incomplete cardiac looping and severely reduced ventricular contractility with disassembled muscular fibers. The high impact of histone acetylation on transcription and on the phenotype is well characterized; e.g., class II histone deacetylases control cardiac growth and gene expression in response to stress stimuli. DPF3 potentially represents the missing link to explain the high impact of the histone modification status on the recruitment of the BAF complex to chromatin target sites and its consequence for cardiac function. The knowledge of the molecular function of Dpf3 contributes to our understanding of cardiac hypertrophy in patients and might provide the basis of future therapeutic options in heart failure.

### C12.2

#### Neuropathology of Alpha-Synuclein and Synphilin-1 transgenic Mouse Models of Parkinson's Disease

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Alpha-synuclein has been implicated in the pathogenesis of many neurodegenerative disorders, including Parkinson's disease (PD). PD is based on progressive neuropathological alterations leading to motor abnormalities that are frequently preceded by olfactory dysfunction

and then cognitive decline in later disease stages. Whether the neurodegenerative process might be halted or even reversed is presently unknown. We therefore generated conditional mouse models by using the tet-regulatable system. Mice expressing high levels of human wildtype alpha-synuclein in several brain regions developed nigral and hippocampal neuropathology, including reduced neurogenesis and neurodegeneration, leading to progressive motor decline and impaired long-term memory. Turning off transgene expression in aged mice halted progression of motor symptoms but did not reverse the symptoms. Mice expressing the mutated (A30P) alpha-synuclein limited to the olfactory bulb showed a reduction of monoamines in this region. In a conducted TMT-smell test these mice also revealed an impaired anxiety reaction and an increased exploratory behavior; the latter might be a depressive core symptom. These data suggest that approaches targeting  $\alpha$ -syn induced pathological pathways might be of benefit only in early disease stages. We further generated mice with permanent expression of the synphilin-1 transgene. We found ubiquitin-positive inclusions in cerebellar Bergman Glia and dark-cell degeneration of Purkinje cells. At the behavioral level mice showed impairment of motor performance and motor skill learning. This data suggest a negative impact of overexpressed synphilin-1 in transgenic mouse brain.

#### C12.3

#### Rescue of a Lethal Murine Model of Methylmalonic Acidemia using rAAV8 Mediated Gene Therapy- One Year Post-Treatment

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Methylmalonic acidemia (MMA) is a genetic disorder caused by deficient activity of methylmalonyl-CoA mutase (MUT). MMA patients exhibit increased methylmalonic acid levels in the plasma and urine and exhibit a clinical phenotype of lethal metabolic decompensation, growth retardation, renal failure and metabolic strokes. A recombinant adeno-associated virus (AAV) serotype 8 carrying the murine *Mut* cDNA (rAAV8-mMut) was injected directly into the liver of newborn *Mut<sup>-/-</sup>* mice. Greater than 95% of the 27 *Mut<sup>-/-</sup>* mice injected with 1 or 2x10<sup>11</sup>GC of rAAV8-mMut have survived for 1 year or longer and are indistinguishable from their control littermates. All the untreated mutants (n=58) perished before day of life 72. Subsequently, a smaller group of *Mut<sup>-/-</sup>* mice (n=4) received an intraperitoneal (IP) injection of 3x10<sup>11</sup>GC of rAAV8-mMut, with 75% of the mice rescued for longer than 120 days. Hepatic *Mut* RNA levels decreased from 37-72% at 90 days post-injection to 10-15% at one year post-treatment. Plasma methylmalonic acid levels in the treated mutant mice were significantly reduced at 24 and 60 days after treatment and remained stable one-year post-treatment, indicating that substantial MUT enzymatic activity was restored and maintained. Whole body MUT enzymatic activity, indirectly measured by *in vivo* conversion of 1-<sup>13</sup>C-sodium propionate into <sup>13</sup>CO<sub>2</sub>, was readily detected in *Mut<sup>-/-</sup>* mice one-year post-treatment. These experiments provide the first evidence that gene therapy has clinical utility in the long term and acute treatment for methylmalonic acidemia and provide proof of principle evidence to support the development of gene therapy for other organic acidemias.

#### C12.4

#### Novel Enzyme Replacement Therapy for Gaucher Disease: On Going Phase III Clinical Trials with Recombinant Human Glucocerebrosidase Expressed in Plant Cells

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Gaucher Disease, characterized by glucocerebrosidase (hGCD) deficiency, provokes glucosylceramide accumulation in cellular lysosomes. Protalix has developed a proprietary plant cell expressed active form of rh-glucocerebrosidase (prGCD). Protalix unique technology permits control of glycosylation pattern and consistency through targeting to specific plant cell organelles. prGCD has intrinsic exposed mannose residues and demonstrates batch to batch consistency. prGCD exhibits similar crystal structure and biological activity to Cerezyme. Preclinical toxicology studies showed no treatment-related adverse events, no neutralizing antibodies and no clinical findings. Phase I safety clinical trial showed that prGCD administered intravenously in sequential doses (15, 30 and 60 units/kg) was well tolerated, all tests being within

normal ranges, with no treatment related adverse events. Pharmacokinetic analysis demonstrated a prolonged half life. All immunological specific tests were within normal ranges with no significant immune reaction or production of anti-prGCD antibodies. An international multi-center Phase III Pivotal trial is currently on-going under FDA SPA approval, where 30 untreated patients are administered with 30U/kg or 60U/kg per infusion over 9 months. Report of the results is expected to take place in H2/2009. Submission of our NDA to the FDA, Israeli and other regulatory agencies will take place in Q4/2009. Protalix has initiated a follow-on extension study with enrollment of our first patients in June 2008. In addition, a multi center worldwide switch-over study to assess the safe transition to prGCD of patients with Gaucher disease that are currently undergoing enzyme replacement therapy with imiglucerase was initiated on December 2008.

#### C12.5

#### Trans-splicing gene therapy in autosomal dominant skin disease

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Mutations in the KRT14 gene underly different types of the blistering skin disease Epidermolysis bullosa simplex (EBS). This form of EB is usually inherited in an autosomal dominant way, rendering it challenge for gene therapy, since the expression of wild type cDNA does not remove the dominant negative keratin aggregates.

We chose "Spliceosome Mediated RNA *Trans-Splicing*" (SMaRT) to repair the heterozygous KRT14 missense mutation R125P. By replacing a specific gene portion by *trans-splicing* of a pre-*trans-splicing* molecule (PTM) to a target pre-mRNA, functionality of the target protein is restored and the amount of negatively interfering dominant gene product is reduced.

Besides the wildtype gene portion to be replaced and essential splicing features, the PTM incorporates a binding domain (BD), which is crucial for the *trans-splicing* specificity and efficiency. We developed a screening method to identify best BDs for a PTM which specifically replaces KRT14 exons 1 to 7. It is based on fluorescent molecules which render successful *trans-splicing* events discriminable.

A PTM library including random binding domains specific for KRT14 exon/intron7 was produced. Transfection of the library with a target molecule results in fluorescence of *trans-spliced* molecules, visible in the fluorescence microscope and detectable by FACS analysis. Functional PTMs were isolated and tested for *trans-splicing* efficiency and specificity. Best PTMs were adapted for endogenous *trans-splicing* and transfected to a EBS keratinocytes cell line, showing specific *trans-splicing* into exon 8 of the endogenous KRT14.

Successful *trans-splicing* in this model proves that this is a novel approach to treat autosomal dominant diseases.

#### C12.6

#### Ciliary beating recovery in deficient human airway epithelial cells after lentivirus ex vivo gene therapy

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Introduction: Primary Ciliary Dyskinesia is a heterogeneous genetic disease which is characterized by cilia dysfunction of the epithelial cells lining the respiratory tracts resulting in recurrent respiratory tract infections. Despite lifelong physiological therapy and antibiotics, lungs of affected patients are progressively destroyed leading to respiratory insufficiency. Recessive mutations in *Dynein Axonemal Intermediate chain type 1 (DNAI1)* gene have been described in 10% of cases of Primary Ciliary Dyskinesia.

Objective: Our goal was to restore normal ciliary beating in DNAI1 deficient human airway epithelial cells.

Methods: A lentiviral vector based on Simian Immunodeficiency Virus pseudotyped with Vesicular Stomatitis Virus Glycoprotein was used to transduce cultured human airway epithelial cells with a cDNA of *DNAI1* driven by the Elongation Factor 1 promoter. Transcription and translation of the transduced gene were tested by RT-PCR and western blot, respectively. Human airway epithelial cells which were DNAI1 deficient due to compound heterozygous mutations and consequently

had immotile cilia and no outer dynein arms, were transduced by the lentivirus. Cilia beating was recorded and electron microscopy of the cilia was performed.

Results: Transcription and translation of the transduced *DNA1* gene were detected in human cells treated with the lentivirus. In addition, immotile cilia recovered a normal beat and outer dynein arms reappeared.

Conclusion: This is the first time that recovery of cilia beating is demonstrated in this disease. This preliminary step constitutes a conceptual proof which is indispensable in the perspective of Primary Ciliary Dyskinesia's *in vivo* gene therapy.

### C13.1

#### Clinical characteristics of distantly related families with idiopathic ventricular fibrillation linked to chromosome 7q36

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Introduction: Idiopathic ventricular fibrillation (IVF) is characterised by VF in the absence of structural or electrical heart disease. With genome wide haplotype sharing analysis we identified an IVF locus on chromosome 7q36. This locus harbors the DPP6 gene and was found in 10 distantly related families with IVF and unexplained sudden cardiac death (SCD).

Methods: We studied echocardiograms, MRIs, ECG characteristics at baseline, exercise and at ajmaline/flecainide provocation, and event-free survival.

Results: We identified 254 relatives, 117 carriers (mean age 43±20 years, 56% males) and 137 noncarriers (mean age 40±21 years, 44% males). Echocardiography (n=33) and MRI (n=43) did not reveal significant or consistent abnormalities. Baseline and exercise ECG characteristics were not different between groups and provocation with ajmaline/flecainide was negative in six resuscitated carriers. SCD/IVF had occurred in 37 carriers (32%, median age 34 years). Kaplan-Meier curves showed severely decreased survival for carriers with only 50% event-free at 60 years of age. Male carriers seemed to be more severely affected with 50% event-free survival at 48 years of age (p=0.004). At present, an implantable defibrillator seems the only treatment option in carriers.

Conclusions: We identified a novel IVF locus on chromosome 7q36 which harbors the DPP6 gene. The associated phenotype is extremely malignant and presents as premature IVF/SCD. Genetic testing for the responsible haplotype is currently the only risk marker in these patients. This raises the unique possibility to assess the risk status of, and treat accordingly, presymptomatic individuals with a potentially fatal disease that does not express otherwise.

### C13.2

#### The yield of family screening in sudden unexplained death in the young

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Introduction: Sudden death of a young person is not explained by autopsy in 6-65%, which is termed sudden unexplained death (SUD). In these cases, molecular autopsy and cardiological and genetic examination in surviving first degree relatives might unmask its cause, especially primary arrhythmia syndromes. We sought to determine the yield of family screening in a large series of young SUD victims.

Methods: At the cardiogenetics department of our university hospital, we studied all consecutive families who presented with ≥1 first degree related SUD victim aged 1-50 years. Autopsy was not performed (53.8%) or did not reveal a cause of death. The initial examination of the relatives consisted of personal and family medical history and a resting ECG. Cardiac autopsy was revised if possible. Additional cardiological examinations were performed on indication. Genetic analysis of the associated candidate gene(s) was performed in material obtained from the deceased person or in those relatives with clinical abnormalities.

Results: The families of 115 SUD victims (mean age at death 29.1

years, 67.8% male) were examined. Per family, a mean of 2.5 (1-8) first-degree relatives were examined (N=242). A (probable) diagnosis was made in 36 of the families (31.3%). Catecholaminergic polymorphic ventricular tachycardia (N=8) and long QT syndrome (N=7) were the most common diagnoses.

Conclusions: By screening family members of a young SUD victim an inherited heart disease is identified in approximately 31% of families. Family screening at a specialized cardiogenetics department should be recommended to relatives of SUD victims.

### C13.3

#### Mutations in the ANKRD1 gene encoding CARP are responsible for human dilated cardiomyopathy

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Dilated Cardiomyopathy (DCM) is familial in about 30% of cases, and to date, 15 responsible genes have been identified in isolated forms and up to 25 associated with additional phenotypes including myopathy, arrhythmias or more complex syndromes. No major gene for the disease has been identified, demonstrating the genetic heterogeneity of DCM. However, in a majority of families the responsible genes are still to be discovered.

The *ANKRD1* gene is overexpressed in heart failure in human or animal models. The encoded protein CARP is interacting with partners such as Myopalladin or Titin, previously involved in DCM. We hypothesised that mutations in *ANKRD1* could be responsible for DCM.

We have screened a DCM affected population consisting on 231 caucasian independent familial (158) and sporadic (73) cases by direct sequencing of PCR-amplified coding exons. We identified 5 missense mutations: 3 sporadic (mutations p.Glu57Gln, p.Arg66Gln and p.Leu199Arg) and 2 familial (mutations p.Thr116Met and p.Ala276Val) absent from 400 controls and affecting highly conserved residues.

Expression of the mutant CARP proteins in rat neonate cardiomyocytes indicated that at least 3 of the mutations identified (p.Glu57Gln, p.Leu199Arg, p.Ala276Val) led both to significant less repressor activity and to greater phenylephrin induced hypertrophy suggesting altered function of CARP mutant proteins. Based on genetic and functional analysis of CARP mutations, we have identified *ANKRD1* as a new gene associated with DCM, accounting for about 4% of cases.

### C13.4

#### Clinical Features and Outcome of Hypertrophic Cardiomyopathy Associated with Triple Sarcomere Protein Gene Mutations

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Double or compound sarcomere gene mutation heterozygosity have been described in 3-6% of hypertrophic cardiomyopathy (HCM) patients, and is associated with early disease onset and severe outcome. In the present study, we provide the first description of the clinical profile associated with triple sarcomere gene mutations in a large, consecutive HCM cohort.

A total of 247 unrelated index HCM patients underwent screening for myofilament gene mutations by automatic DNA sequencing of eight genes: MYBPC3, MYH7, TNNT2, MYL2, MYL3, TNNI3, TPM1, and ACTC.

Of the 247 index patients, 3 (1%) harboured triple gene mutations, as follows: MYH7-R869H, MYBPC3-E258K and TNNI3-A86fs in a 32-year old female; MYH7-R273C, MYH7-E1455X and MYBPC3-E165D in a 46-year old male; and MYBPC3-insC1065, MYBPC3-P371R and MYH7-R869H in a 45-year old female. All 3 experienced early onset of severe HCM phenotype, marked symptoms and progression of disease towards the end-stage phase by the fourth decade, requiring, in one instance, cardiac transplantation. Moreover, all had multiple risk factors for sudden cardiac death, including resuscitated cardiac arrest

in one, advising the implantation of prophylactic defibrillators which appropriately intervened in two patients. Each of the probands' parents was a documented or likely carrier of one or two mutations. However, none had a prior diagnosis of HCM or significant cardiac symptoms. HCM associated with triple gene mutations was very rare but invariably associated with rapidly progressive disease and adverse outcome. These findings reinforce the unfavourable prognostic significance of complex genotypes in patients with HCM, with potential impact on genetic screening strategies.

### C13.5

#### Noncompaction Cardiomyopathy; mutation spectrum, distribution of disease genes and implications for diagnostic strategies

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**Background:** Noncompaction cardiomyopathy (NCCM) is characterised by an excessively thickened endocardial layer with deep intertrabecular recesses. Cardiac symptoms include heart failure, lethal arrhythmias and/or thrombo-embolic complications. NCCM is genetically heterogeneous, predominantly autosomal dominantly inherited. To contribute to a genetic classification 17 different genes associated with cardiomyopathy were completely analysed in a cohort of 56 NCCM patients. **Methods:** DNA analysis of the genes  $\beta$ -Myosin Heavy Chain (*MYH7*), Myosin Binding Protein C (*MYBPC3*), cardiac Troponin C (*TNNC1*), Troponin T (*TNNT2*) Troponin I (*TNNI3*) and  $\alpha$ -Tropomyosin (*ACTC1*), cardiac-regulatory Myosin Light Chain (*MYL2*), cardiac-essential Myosin Light Chain (*MYL3*),  $\alpha$ -Tropomyosin (*TPM1*), Cysteine- and Glycine-rich Protein (*CSRP3*), Thelenitin (*TCAP*), Calsequestrin (*CASQ2*), Calreticulin (*CALR3*), Phospholamban (*PLN*), Taffazin (*TAZ*), Cypher/Zasp (*LDB3*) and Lamin A/C (*LMNA*) was performed.

**Results:** Twenty-nine mutations were identified in the genes *MYH7* (11), *MYBPC3* (4), *TNNT2* (3), *TNNI3* (1), *TPM1* (2), *ACTC1* (1), *CASQ2* (2), *PLN* (1), *TAZ* (1), *LDB3* (2) and *LMNA* (1) in 23 probands (41%). Eighteen probands had a single mutation, four had two and one had three mutations.

**Conclusion:** We identified the *MYBPC3*, *TNNI3*, *TPM1*, *PLN* and *CASQ2* genes as novel NCCM loci. In 41% of the patients NCCM was associated with mutations in sarcomere, Z-disc,  $\text{Ca}^{2+}$ -handling or other genes associated with cardiomyopathy. This warrants molecular analysis of these genes in NCCM. The identification of the genetic cause for cardiomyopathy facilitates family study and allows accurate identification of relatives at risk of developing cardiomyopathy. Genetically, NCCM is part of a continuous pathophysiological spectrum including hypertrophic, dilated and restrictive cardiomyopathy.

### C13.6

#### Common variants at ten loci modulate the QT interval duration in individuals of European ancestry: the QTSCD consortium

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The QT interval, a measure of cardiac repolarization, predisposes to ventricular tachycardia and sudden cardiac death (SCD) when pro-

longed or shortened. Previously, a common variant in NOS1AP (CAPON) influencing QT interval was mapped in a European population. We now analyze genome-wide association data from five European ancestry samples (ARIC, KORA, SardiNIA, GenNOVA and HNR, N = 15,842). We confirm the NOS1AP association ( $P=1.63\times 10^{-35}$ ) and identify nine additional loci at  $P<5\times 10^{-8}$ . Four loci map near the monogenic long QT syndrome genes KCNQ1, KCNH2, SCN5A and KCNJ2. Two loci map to ATP1B1 ( $P=2.18\times 10^{-12}$ ), PLN ( $P=1.97\times 10^{-16}$ ) that have well known roles in myocardial electrophysiology. The remaining loci are at RNF207 ( $P=3.57\times 10^{-9}$ ), LITAF ( $P=2.92\times 10^{-8}$ ) and near GINS3-NDRG4-CNOT1 ( $P=1.26\times 10^{-12}$ ). Taken together genetic variation at these 10 loci explained 3.3% of corrected QT interval variation across all studies. The ~8% of individuals carrying 14 or more QT prolonging alleles had an OR of 2.52 to have prolonged QT by clinical standards when compared to the ~10% of individuals carrying 8 or less alleles (95% CI 1.74-3.66,  $P=4.83\times 10^{-7}$ ). These results provide new insights into myocardial electrophysiology and provide novel candidate genes for ventricular arrhythmias and SCD.

### C14.1

#### The vertebrate inner ear microRNAs have unique spatial and temporal expression patterns and are crucial for inner ear development and survival

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MicroRNAs (miRNAs) regulate the translation of target mRNAs and affect, directly or indirectly, the expression of a large portion of the protein-coding genes. The RNase Dicer1 is required for production of mature and functional miRNAs from pre-miRNAs. The mammalian inner ear contains the cochlea and the vestibule that are responsible for hearing and balance, respectively. *Dicer1* was conditionally knocked-out in the sensory epithelia of the mouse inner ear, to study the roles of miRNAs in these tissues. Mutant mice were born with normal sensory hair cells, but developed malformed hair cells and profound deafness a short time thereafter, demonstrating that miRNAs are crucial for postnatal survival of functional inner ear hair cells. We identified miRNAs that may have a role in the mouse developing inner ear by combining miRNA transcriptome analysis and bioinformatics. Different spatial and temporal expression patterns of six miRNAs (miR-15a, miR-18a, miR-30b, miR-99a, miR-182 and miR-199a) may reflect their roles. A subset of these miRNAs, miR-15a, miR-18a and miR-30b, were shown to be crucial for early development and morphogenesis of the zebrafish inner ear. To search for putative target mRNAs for miRNAs that are expressed in the mouse inner ear sensory epithelia, we intersected the outputs of target prediction algorithms with the mRNA expression profiles of these tissues. *Slc12a2*, *Cldn12* and *Bdnf* mRNAs were examined as targets for miR-15a. Our data support the hypothesis that inner ear tissue differentiation and maintenance are regulated and controlled by conserved sets of cell-specific miRNAs in both mouse and zebrafish.

### C14.2

#### Molecular basis of alpha-dystroglycanopathies and phenotype-genotype correlations

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The alpha-dystroglycanopathies are a group of autosomal recessive congenital muscular dystrophies (CMD) and limb girdle muscular dystrophies (LGMD) with high CK levels, muscle hypertrophy, and

abnormal glycosylation of  $\alpha$ -dystroglycan in muscle. Only a portion of the patients present with mental retardation (MR), with or without structural brain and ocular malformations. These disorders are caused by mutations in at least six genes encoding O-mannosyltransferases (*POMT1* and *POMT2*), O-mannosyl N-acetylglucosaminyltransferase1 (*POMGNT1*), and enzymes of unknown function (*FKRP*, *LARGE* and *FKTN*).

The most frequently mutated gene is *FKRP*, associated with a large spectrum of phenotypes varying from mild LGMD2I often due to the founder mutation, L276I, to the most severe form of the spectrum with major structural brain and eye malformations and early lethality, called Walker Warburg syndrome (WWS). We studied a large cohort of muscular dystrophy patients with histological and clinical features of  $\alpha$ -dystroglycanopathy and identified mutations in all 6 genes, including founder mutations in *FKRP* and *POMT2*, and large genomic rearrangements in *POMT2* and *LARGE*.

From genotype-phenotype analysis of 26 CMD families with alpha-dystroglycan hypoglycosylation and *POMGNT1* (3), *POMT1* (4), *POMT2* (10), *FKTN* (3), and *LARGE* (1) mutations, we suggest the screening of *POMT1* and *POMT2* first in CMD patients with MR, especially if there is microcephaly, cerebellar hypoplasia, white matter abnormalities or cortical dysplasia, even in the absence of eye involvement. In CMD or LGMD patients with normal intellectual development, other genes are better candidates (*FKRP*, *FKTN*), especially if there is progressive cardiac dysfunction.

#### C14.3

##### Mutations of *LRTOMT*, a fusion gene with alternative reading frames, cause autosomal recessive nonsyndromic hearing impairment in *DFNB63* families.

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Heredity hearing impairment is a genetically heterogeneous disorder. Identification of the causative deafness genes is a considerable strategy to uncover the molecular basis of hearing. Reassessment of three *DFNB63* families which were used in the identification of the *DFNB63* locus and two additional Tunisian families helped to narrow the critical *DFNB63* region to a 1.03 Mb interval. This interval has 26 annotated and predicted genes and sequencing of these genes revealed four pathogenic mutations in an uncharacterized gene *LRRC51*, renamed *LRTOMT*. *LRTOMT* has two alternative reading frames and encodes two different proteins *LRTOMT1* and *LRTOMT2*. *LRTOMT2* is a putative methyltransferase. Further characterization showed that in the primate lineage, *LRTOMT* evolved from the fusion of two neighboring ancestral genes. In rodents, there are two separate genes, designated *Lrrc51* and *Tomt* which together are orthologous to the primate *LRTOMT*. RT-PCR analysis of human tissues showed that *LRTOMT* is widely expressed. RNA *in situ* hybridization in mouse revealed the expression of *Tomt* in the cochlear and vestibular sensory cells of the developing inner ear. Immunolocalization studies of *Lrrc51* and *Tomt* in the P30 mouse inner ear showed the expression of both genes in

vestibular and cochlear hair cells and their supporting cells. Our data indicates that *LRTOMT* is essential for hearing and the identification of *LRTOMT*, which encodes a leucine-rich protein and a methyltransferase, opens an exciting new field for genetic and physiological studies of the inner ear and hearing.

#### C14.4

##### Clinical and mutational spectrum of the Legius syndrome (or NF1-like syndrome)

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Autosomal dominant inactivating *SPRED1* mutations have recently been described in 5 families with a neurofibromatosis type 1-like syndrome (NFLS). The phenotype consists of café-au-lait-macules (CALM), axillary freckling and macrocephaly. The full clinical spectrum of this new disorder has not yet been investigated.

We performed *SPRED1* mutation analysis in 1318 unrelated patients presenting with a broad range of signs typically found in neurofibromatosis type 1 (NF1) and in whom no *NF1* mutation was present in peripheral blood lymphocytes. A comparison between clinical findings in patients with a *SPRED1* mutation versus those with a *NF1* mutation and those without known mutation was made. Functional assays were used to evaluate the pathogenicity of identified missense mutations. We identified 34 different *SPRED1* mutations in 43 probands: twenty seven were pathogenic (including 2 missense mutations) and 7 missense mutations were classified as probably benign. Forty eight percent of *SPRED1*-positive patients fulfilled NIH NF1 diagnostic criteria based on the presence of >5 CALM +/- freckling or a NF1-compatible family history. We estimate that 1.2-2.9% of individuals with the clinical diagnosis of NF1 have NFLS.

A high *SPRED1* mutation detection rate was found in *NF1* mutation-negative families with an autosomal dominant phenotype with CALM +/- freckling and no other NF1 features. The NF1 diagnostic criteria are not specific, since 48% of patients with NFLS fulfilled these criteria. NFLS is not associated with the high incidence of peripheral and central nervous system tumors seen in NF1. We suggest a less intensive medical follow-up program for patients with NFLS.

#### C14.5

##### Three subgroups of neurodegeneration with brain iron accumulation (NBIA)

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Objective: Neurodegeneration with brain iron accumulation (NBIA) is a heterogeneous group of disorders characterized by iron deposits in the basal ganglia. Mutations in two different genes can be responsible for NBIA: *PANK2* gene and *PLA2G6* gene. A third gene was discovered recently by our laboratory. From the clinical point of view all NBIA patients share common symptoms. Nevertheless we suppose these patients can be distinguished in three clinical subgroups. Because of small patient numbers analysis of clinical symptoms is difficult.

Methods: We present a structured clinical evaluation of a large collection of NBIA patients characterized by one clinician (n=49). A mutation analysis of the *PANK2* gene has been done in all patients. Patients

being negative for *PANK2* mutations have been analysed on *PLA2G6* gene and furthermore on the recently detected gene.

**Results:** In 27 out of 49 patients homozygous or compound heterozygous mutations were identified in *PANK2* (55%). No *PLA2G6* mutations were found. Mutations in the new gene were detected in 16 patients (33%). The "eye of the tiger" sign in MRI was found in the majority of patients with *PANK2* mutations (24 out of 27) but rarely in the remaining patients. Furthermore patients with *PANK2* mutation, with mutations in the new gene and idiopathic NBIA patients can be distinguished by several clinical features: age of onset, generalized dystonia as presenting symptom, course of disease, oromandibular dystonia, optic atrophy and psychiatric symptoms.

**Conclusion:** Clinical symptoms and MRI together allow us to distinguish NBIA patients in a first step.

#### C14.6

#### Congenital Disorders of Glycosylation (CDG) due to defects in N-glycan assembly in the endoplasmic reticulum: filling the gaps in the dolichol cycle

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Congenital disorders of glycosylation (CDG) are a group of complex metabolic diseases caused by defects in the synthesis, assembly and processing of glycans. Through EUROGLYCANET, we have access to a collection of 50 unsolved CDG-I patients (CDG-Ix). An investigation of lipid-linked oligosaccharides (LLO) has been performed on all cases. We report a patient with severe developmental delay, epilepsy and dysmorphic features, and a type I pattern on transferrin isoelectric focusing. LLO showed an accumulation of the dol-PP-GlcNAc2-Man5 structure, and a limited and very unusual accumulation of the dol-PP-GlcNAc2-Man3, dol-PP-GlcNAc2-Man6, dol-PP-GlcNAc2-Man7 and dol-PP-GlcNAc2-Man8 species.

This patient was compound heterozygous for 2 mutations in the DPM2 gene: a splice mutation (IVS2-1G>C) and a point mutation (c.68A>G, p.Y23C). DPM2 is the smallest component of the dolichol-phosphate-mannose synthase and is a hydrophobic protein of 84 amino acids. It seems that DPM2 is involved in the regulation of the DPM synthase complex by stabilizing DPM3 but also in the regulation of the GPI-GnT (glycosylphosphatidylinositol-N-acetylglucosaminyltransferase). We also identified, among other new cases, 2 new patients with an RFT1 deficiency, respectively homozygous for the missense mutations c.454A>G (p.E152K) and c.892G>A (p.K298E). The clinical phenotype includes severe mental retardation, failure to thrive, hypotonia, epilepsy, sensorineural deafness, coagulopathy and feeding problems. The transmembrane protein RFT1 is suggested to play a crucial role in the flip-flop of the intermediate Man5GlcNAc2-PP-dolichol from the cytosolic to the luminal face of the ER membrane. Flipping of lipids across biomembranes is not spontaneous and requires specific proteins (flippases) that facilitate ATP-independent, bi-directional flip-flop.

#### C15.1

#### Segmental copy number variation shapes tissue transcriptomes

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Copy number variation (CNV) of DNA segments has recently been identified as a major source of genetic diversity, but a comprehensive understanding of the phenotypic effect of this type of variation is only beginning to emerge. We have generated an extensive map of CNV in wild mice and classical inbred strains. Copy number variable regions cover a total of ~340 megabases (~11%) of their autosomal genome. Genome-wide expression data from 6 major organs and 4 developmental times in six different strains reveal that expression levels of genes within CNVs positively or negatively correlate with copy number changes in approximatively 35 and 15% of the cases, respectively. Our

experiments also show that CNVs influence the expression of genes in their vicinity - an effect that extends up to half a megabase. These controls over expression are effective throughout mouse development, however some genes appear to be under compensatory loops at specific time point.

Interestingly, genes within CNVs show lower expression levels and more specific spatial expression patterns than genes mapping elsewhere in the genome. Furthermore, genes expressed in the brain are significantly underrepresented in CNVs compared to genes with expression in other tissues, suggesting differential selective constraint on copy number changes of genes expressed in different tissues. Our study provides initial evidence that CNVs shape tissue transcriptomes on a global scale and thus represent a significant source for within-species phenotypic variation.

#### C15.2

#### Methylation profiling in cases with uniparental disomy identifies novel imprinted genes on chromosome 15

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One of the major features associated with imprinting is the presence of parent-of-origin specific Differentially Methylated Regions (DMRs). Thus, the maternal and paternal genomes possess distinct epigenetic marks which distinguish them at imprinted loci. In order to identify novel imprinted genes on chromosome 15, we have profiled DNA methylation in cases with uniparental disomy of chromosome 15 (UPD15). Methylated DNA was immunoprecipitated using antibodies for 5-methyl cytidine, and hybridized to high-density oligonucleotide arrays with complete coverage of chromosome 15, generating profiles of the paternal and maternal methylomes. Comparison of six individuals with maternal versus paternal UPD15 reveals more than twenty DMRs on chromosome 15. Putative DMRs were validated by bisulfite sequencing, confirming the presence of parent-of-origin specific methylation marks in multiple samples. Many are associated with known imprinted genes within the Prader-Willi/Angelman syndrome region, such as *SNRPN* and *MAGEL2*, validating this as a method of detecting imprinted loci. However, more than half of the novel DMRs identified are located outside of 15q11-q13, and are associated with genes not previously thought to be imprinted. These include *IGF1R* at 15q26.3, which plays a fundamental role in growth regulation, and *GABRG3*, a gene which has previously been shown to be abnormally expressed in autism. Many DMRs occur at CpG islands or overlap conserved non-coding regions, suggesting a role in regulating gene expression. These data provide an imprinting map of chromosome 15, demonstrate that the number of imprinted loci in humans is much higher than previously thought, and identify novel candidates for human disease.

#### C15.3

#### Closely spaced multiple mutations as potential signatures of transient hypermutability in human genes

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Data from a diverse series of organisms suggested that transient hypermutability is a general mutational mechanism with the potential to generate multiple synchronous mutations, a phenomenon probably best exemplified by closely spaced multiple mutations (CSMMs). However, to date, its potential impact on the evolution of higher eukaryotes has not been widely appreciated. Here we attempted to extend the concept of transient hypermutability from somatic cells to the germline, using human disease-causing multiple mutations as a model system. Employing fairly stringent criteria for data inclusion, we have retrospectively identified numerous potential examples of CSMMs causing human inherited disease that exhibit marked similarities to the CSMMs reported in other systems. These examples include (i) eight multiple mutations, each comprising three or more components within a sequence tract of <100 bp, (ii) three possible instances of 'mutation

showers' and (iii) numerous highly informative 'homocoordinate' mutations. Using the proportion of CpG substitution as a crude indicator of the relative likelihood of transient hypermutability, we also present evidence to suggest that CSMMs comprising at least one pair of mutations separated by <100 bp may constitute signatures of transient hypermutability in human genes. This analysis not only extends the generality of the concept of transient hypermutability but also provides new insights into what may be considered a novel mechanism of mutagenesis underlying human inherited disease. Finally, our findings raise serious concerns regarding current practices in mutation screening, which are likely to miss many potentially important secondary mutations linked in cis to the putative primary pathological lesion.

#### C15.4

##### The Human Phenotype Ontology

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There are many thousands of hereditary diseases in humans, each of which has a specific combination of clinical features. We have developed a Human Phenotype Ontology (HPO) with over 9500 terms representing individual phenotypic anomalies and have annotated all clinical entries in Online Mendelian Inheritance in Man with the terms of the HPO.

Each term in the HPO describes a phenotypic abnormality such as Atrial septum defect. The HPO itself does not describe individual disease entities but rather the phenotypic abnormalities associated with them. Clinical entities are annotated to the most specific terms possible. The true path rule applies to the terms of the HPO. That is, if a disease is annotated to the term Atrial septal defect, then all of the ancestors of this term, such as Abnormality of the cardiac septa, also apply. The structure of the HPO therefore allows flexible searches for disease entities according to phenotypic abnormalities with a broad or narrow focus.

We have used the HPO to identify a phenotypic network that is made up of dense clusters of shared phenotypic features that show characteristic patterns of interconnections between selected areas of the phenotypic continuum. We have developed a program for clinical genetics diagnostics, which allows searches for specific diagnoses based on combinations of features. We have extended our methods for prediction of disease genes based on random walk analysis to work for arbitrary phenotypes based on ontological similarity analyses using the HPO.

The HPO is freely available at <http://www.human-phenotype-ontology.org>.

#### C15.5

##### GEN2PHEN: An international effort to optimise and federate the databasing of gene-disease relationships

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The GEN2PHEN consortium (<http://www.gen2phen.org>), which comprises 19 research and company partners, is devising novel strategies, standards and databases, to ensure that genotype-phenotype (G2P) information can be shared and exploited effectively. Our activities are based on the idea that rather than just centralising G2P data, the broad community should also be involved in providing G2P databases and analysis platforms, as a part of a federated system. We therefore produce components to help this federated system emerge, in partnership with projects such as ENGAGE, CASIMIR, EUROGENTEST, BBMRI, ELIXIR, and the HVP.

GEN2PHEN has already achieved considerable progress, as reflected by: 1) producing the Locus Reference Genomic (LRG) system of stable reference sequences for genes [in partnership with NIH]; 2) producing the Phenotype and Genotype Experiment Object Model (PaGE-OM) standard data model for G2P information [in partnership with JBIGC];

3) producing new or improved versions of genome wide databases, including HGvbaseG2P (<http://www.hgvbaseg2p.org>), IGVdb (<http://www.igvdb.res.in>), and Disease Cards (<http://bioserver.ieeta.pt/diseasercard>);

4) producing improved versions of locus specific database (LSDB) software LOVD (<http://www.lovd.nl/2.0>) and UMD (<http://www.umd.be>), that now host >300 genes, and the DMuDB diagnostic database (<http://ngrl.man.ac.uk/dmudb>)

Our current focus extends into areas such as grid-based services, and systems for providing universal IDs for all biomedical research data and entities on the internet, including individual researchers. This will revolutionise the potential for holistic data integration, and ensure fair and equitable use of sensitive G2P information.

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#### C15.6

##### Establishing a link between microRNAs, genes and hereditary diseases : the miRiFix database

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Identification and analysis of microRNAs (miRs) enhance our understanding of the important roles that small RNAs play in complex regulatory networks. However, there are still few data supporting the involvement of miRs in Mendelian disease inheritance other than cancer. MiRs may be regarded either as candidate gene within a disease locus, or as putative modifier gene, which can regulate the expression of a given disease-causing gene. At this latter level, single nucleotide polymorphisms (SNPs) within the target gene add a supplemental layer of complexity. Herein, we present a comprehensive resource, aimed at linking miRs and hereditary diseases : mirifix.com. MiRiFix is an easy-to-use, web-accessible framework of tool and data integration. Our model crosses up-to-date information on human miRs and the Genatlas database, which provides integrated data on gene mapping and genetic diseases. MiRiFix enables to systematically explore the computational involvement of miRs in the pathogenesis of diseases, and retrieves : (i) miR as a candidate gene from a locus, using the updated compendium of human miRs and their mapping information, (ii) a set of miRs predicted to regulate a disease-causing gene, in both its 3'-UTR and coding sequence, using distinct algorithms, and finally (iii) a set of SNPs predicted to be functional in terms of miR regulation. We will present the efficiency of miRiFix in predicting previously established links, but also in retrieving novel data on mapped diseases orphan of identified genes. Our web resource provides a unique integrated way to assess computational roles of miRs in hereditary disease.

#### C16.1

##### The Effect of Translocation-Induced Nuclear Re-organization on Gene Expression

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Chromosome organization in the nucleus is thought to impact on gene expression. To study the effect of balanced chromosomal rearrangements on gene expression, we compared the transcriptomes of cell lines from control and t(11;22)(q23;q11) individuals. This translocation between chromosomes 11 and 22 is the only recurrent constitutional non-Robertsonian translocation in humans. The number of differentially expressed transcripts between the translocated and control cohorts is significantly higher than that observed between control samples alone, suggesting that balanced rearrangements have a greater effect on gene expression than normal variation. Altered expression on translocation chromosomes is limited to chromosome 11-mapping genes. Consistently, we show that the nuclear position of the derivative chromosome 11, but not that of the derivative chromosome 22, is significantly altered compared to its normal counterpart, suggesting that expression changes of chromosome 11 genes are potentially due to their transposition into an anomalous chromatin environment. Our

results are consistent with recent studies, which indicate that nuclear position plays a functional role in regulating the expression of some genes in mammalian cells. Rearrangements may also have implications on reproductive separation, as we show that reciprocal translocations not only provide partial isolation for speciation, but also result in significant changes in transcriptional regulation through alteration of the relative nuclear position of chromosome territories.

#### C16.2

#### 5q14.3 microdeletion encompassing *MEF2C*, a gene controlling excitatory synapse number, is associated with severe mental retardation, poor eye contact and seizures

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Over the last few years, array-CGH has remarkably improved the ability to detect cryptic unbalanced rearrangements in patients presenting with syndromic mental retardation. Using oligonucleotide array-CGH, we identified a 5q14.3 microdeletion in 5 unrelated patients and a 5q14.3 microduplication in one patient. Fluorescence *In Situ* Hybridization (FISH) and semi-quantitative PCR further confirmed these *de novo* rearrangements. Interestingly, the five patients with the 5q14.3 microdeletion showed striking phenotypic similarities, namely severe mental retardation, poor visual contact, absent speech, stereotyped behaviour, facial dysmorphic features, epilepsy and/or cerebral malformations. The boundaries and sizes of the deletions were different but the minimal common deleted region encompassed only the *MEF2C* gene. This gene, known to act in brain as a neurogenesis effector which regulates excitatory synapse number, constitutes an excellent candidate. We suggest therefore that 5q14.3 microdeletion may represent a novel clinically recognizable condition caused by haploinsufficiency of the *MEF2C* gene.

#### C16.3

#### 19q13.11 deletion syndrome: a novel clinically and biochemically recognizable genetic condition identified by array-CGH

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Deletions of chromosome 19 have rarely been reported with the exception of some patients with deletion 19q13.2 and Blackfan-Diamond syndrome due to haploinsufficiency of the RPS19 gene. The paucity of observations might result from the difficulty to detect small rearrangements on this chromosome that lacks a clear banding pattern. Using array-based comparative genomic hybridization (array-CGH) we identified three distinct but overlapping interstitial 19q13.11 deletions, defining a minimal critical region of 2.87 Mb, associated with a clinically recognizable syndrome. The three patients share several major features including: pre and postnatal growth retardation with slender habitus, severe postnatal feeding difficulties, microcephaly, hypospadias, signs of ectodermal dysplasia and cutis aplasia over the posterior occiput. Among the deleted genes, the human prolidase

(PEPD) gene is of particular interest. It is involved in a rare autosomal recessive disorder of the connective tissue. Biochemical assays showed reduced prolidase activity in our patients. Their dermatological anomalies, including cutis aplasia of the scalp and thin skin, might thus be accounted for by PEPD haploinsufficiency. We suggest that del 19q13.11 represents a novel clinically recognizable microdeletion syndrome caused by haploinsufficiency of dosage sensitive genes in the 19q13.11 region. Moreover, as a complement to the specific clinical features, prolidase activity in erythrocytes may provide a simple diagnostic marker for this new genomic disorder.

#### C16.4

#### Parental origin and possible mechanisms of formation of de novo balanced reciprocal translocations

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Balanced reciprocal translocations are found in approx. 1 : 400 newborns. Most of them are inherited from one parent. *De novo* formation is a rare event and accompanied by various anomalies if there is a microrearrangement closed to one or both breakpoints. From associated deletions or duplications, the involvement of the Y chromosome, or cytogenetic polymorphisms a preferentially formation in paternal meiosis was assumed but so far not proven directly.

Here, we report on the parental origin and possible mechanisms of formation of *de novo* cytogenetically balanced reciprocal translocations in two healthy probands and seven patients affected by multiple congenital anomalies. Two of them have been recorded previously. The karyotypes are 46,XY,t(4;5)(q21.1;p15.33), 46,XX,t(2;3)(q33;q23), 46,XY,t(6;14)(q15;q24), 46,XX,t(2;8)(p13~15;q22), 46,XX,t(2;5)(p21;q11.2), 46,XX,t(2;13)(p13;q12), 46,XY,t(7;11)(q1.2;1;p11.2), 46,XX,t(2;7)(q23;p21), and 46,XX,t(2;4)(p16;q35).

For each chromosome of interest, microdissected derivative chromosomes and their normal homologs were pooled separately for investigation, which included whole genome amplification (GenomePlex Single Cell Kit®, Sigma-Aldrich, Vienna, Austria), microsatellite mediated haplotype analysis, and visualisation of the products by silver staining subsequent to a 6% polyacrylamide/urea gel electrophoresis.

Involvement of paternal chromosomes was found in all seven cases investigated so far. Our results confirm the assumed preferentially paternal origin of *de novo* reciprocal translocations for the first time by direct investigation of the chromosomes involved. In addition, the conformity for either maternal or paternal origin for all derivative chromosomes and their normal homologs makes a meiotic formation more likely than a postzygotic formation.

#### C16.5

#### 22q13.3 deletion syndrome is a multigenic disorder, with *SHANK3* as the major pathogenic gene

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The 22q13.3 deletion syndrome consists of hypotonia, mental retardation with delayed or absent speech, normal or accelerated growth, autistic-like behaviour and few dysmorphic features. This condition was recently inferred to be a single gene (*SHANK3*) disorder.

We analysed a total of 32 patients presenting with clinical manifestations consistent with this condition, by means of a-CGH, FISH with cosmids n66c4 and n85a3 containing *SHANK3* and sequence analysis of *SHANK3*. Loss-of-function mutations limited to *SHANK3* were detected in three patients (9%). They were c.3895delG (p.Glu1299fs)dn,

partial gene deletion (n66c4 x 2, n85a3 -) dn, and c.1349T mat. Patients with *SHANK3* mutation (Group A) were compared with a total of 7 patients (group B) with a true 22q13 deletion, varying in size from 1.4 to 7 Mb. Briefly, patients in group A and B shared accelerated growth, absent/severely retarded speech, facial coarseness and hypotonia. All the patients in group A had familiarity for behaviour disorders and additional CNVs. On the contrary, patients in group B were all sporadic with respect to behaviour disorders, and no additional CNVs were detected. Interestingly, the mother with c.1349T mutation presented with behaviour abnormalities but with normal intelligence and without additional clinical manifestations of the 22q13 deletion syndrome. Different CNVs outside 22q13.3 were present in the remaining parents with behaviour abnormalities but with normal *SHANK3*. We suggest that the 22q13 deletion syndrome is unlikely to be a single gene disorder. Although *SHANK3* can be considered the major pathogenic gene, additional gene mutations are requested for the full phenotype.

#### C16.6

#### An International Standardized Cytogenomic Array (ISCA) Consortium approach to the design, implementation and reporting of constitutional oligo array-CGH

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Experience to date in ~1,000 reported cases from the National Genetics Reference Laboratory (Wessex) in the UK has shown that use of a customized 4x44k oligo array in karyotypically normal patients ascertained for developmental delay or mental retardation with or without congenital abnormalities, results in the detection of ~25% significant copy number changes (CNCs) ~15% of which are *de novo*. The NGRL (Wessex) is also part of the ISCA Consortium which is a collaboration comprising ~70 laboratories in the USA, Canada, South America and Europe. Two ISCA workshops held in 2008 led to agreement in two main areas firstly the design, testing and implementation of oligo arrayCGH utilising multiple formats including 8x60k, 2x105k and 4x180k. The consensus design is based on extensive experience derived principally from five laboratories each of which independently custom designed constitutional cytogenetic arrays (4x44k or 2x105k) using Agilent's eArray software. The ISCA oligo array combines targeting for ~500 genomic regions of known or suspected pathogenicity together with an "even backbone" coverage of one oligo probe every ~25 kb. The second ISCA objective is to set up genome browser-based data sharing between Consortium Laboratories to help develop data sets which will help to differentiate between benign and pathogenic copy number changes. The NGRL (Wessex) is about to initiate testing of the ISCA 4x180k array and preliminary results on 40 patients together with a summary of our results to date with the 4x44k array will be presented.

#### C17.1

#### Genome-wide analysis in Parkinson's disease

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Parkinson's disease (PD) is a progressive, age-associated neurodegenerative disorder characterized by loss of dopaminergic neurons, resting tremor, bradykinesia, rigidity, and postural instability. PD, which is estimated to be the second most common neurodegenerative disease, will become an increasing social and economic burden as the world population continues to live longer. In an attempt to further understand the genetic basis of PD and thus gain insight into the etiology of this disorder we performed a genome wide association study in cohorts of Caucasian (n = 5,691) and Asian (n = 3,500) PD cases and controls. Replication of the most significant *loci* was performed in

a total of 7,745 Caucasian and ~ 4,000 Asian cases and controls. The strongest consistent association across all populations was observed within the gene encoding alpha-synuclein (Asian, rs11931074, odds ratio (OR) = 1.2, p = 6.17 x 10<sup>-13</sup>; Caucasian, rs2736990, OR = 1.27, p = 5.69 x 10<sup>-9</sup>), providing unequivocal evidence that common variation at this *locus* is a risk factor for typical PD. A Caucasian-specific association was identified across the MAPT locus (rs415430, OR = 1.34 ; p = 4.5x10<sup>-8</sup>), not only indicating that variation at this locus, implicated in several neurodegenerative disorders, is a risk factor for PD but also that the well-known population specific heterogeneity across this genomic region impacts risk for disease.

Besides, our analysis suggests that a 0.17Mb region in chromosome 1q32 containing 7 genes and predicted transcripts might confer risk for the development of PD in both the Asian and the Caucasian cohorts.

#### C17.2

#### A regional high risk isolate for schizophrenia reveals an enrichment of three large copy number variations overlapping developmental genes

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Copy number variations (CNVs) have consistently been reported to occur more frequently in patients with schizophrenia compared to healthy individuals. Already several rare CNVs, suggestive of high risk for the disease, have been identified. We analyzed a special Finnish study sample of 196 schizophrenia cases and 199 controls emerging from a high risk isolate for schizophrenia with a specific interest to identify potential moderate to high risk CNV alleles that thanks to recent population bottle necks would have become enriched in this founder population. We discovered three large (> 50 kb) CNV alleles on chromosomes 9p24.3, 17p13.3, and 22q11.22 significantly enriched to isolate schizophrenia cases (p < 0.05). After analyzing additional 4,431 Finnish population controls, as well as 2,614 schizophrenia cases and 42,276 controls of European origin, the three CNVs were found to be significantly enriched in the Finnish sub-isolate (p < 1.5\*10<sup>-6</sup>) and within the isolate associating to schizophrenia (p < 0.05). The deletion on 22q11.22, yielding the strongest association with schizophrenia, was found to be 3-fold more frequent in families with schizophrenia compared to the general population of the isolate (15% vs. 3%, p = 1.2\*10<sup>-7</sup>). The CNVs harbor three developmental genes: *DOCK8*, *ABR*, and *TOP3B*, not previously linked to schizophrenia. Our finding refers to the impact of population bottleneck on the enrichment of sizable CNVs overlapping developmental genes and implies their role in the etiopathogenesis of familial schizophrenia. Our finding also exemplifies the importance of region- or even family specific high risk variants behind schizophrenia.

#### C17.3

#### Twenty-two Loci affecting haematological traits

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Haematological traits are the most commonly measured blood parameters in clinical practice and impact on a wide range of disorders including cancer, cardiovascular and immune diseases. We carried out a meta-analysis of genome wide data on eight haematological traits - red blood cell, white blood cell and platelet counts, red blood cell volume, mean platelet volume, mean corpuscular hemoglobin content and mean corpuscular hemoglobin concentration - in 4,627 individuals from three population-based cohorts, followed by replication in 9,515 individuals from three additional population-based samples. We describe 22 genetic loci, of which 15 are novel, associated with one

or more traits at the genome wide significance threshold of  $5 \times 10^{-8}$ . These include 6 loci affecting erythrocyte traits (e.g. HFE, P-value =  $1.4 \times 10^{-23}$ , TFR2, P-value =  $4.9 \times 10^{-10}$ , HBS1L-MYB, P-value =  $7.4 \times 10^{-42}$ ), one locus for leukocyte count, 11 loci for mean platelet volume (e.g. ARHGEF3, P-value =  $5.5 \times 10^{-31}$  and TAOK1, P-value =  $1.4 \times 10^{-22}$ ) and 3 loci for platelet count. The findings identify novel regulators of hematopoiesis in humans, and provide new insights into functional networks regulating blood cell parameters.

#### C17.4

#### The association of Bardet-Biedl Syndrome and Hirschsprung Disease highlights the role of the primary cilium in ENS development

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Isolated Hirschsprung disease (HSCR) is a model for multigenic mode of inheritance. Some chromosomal rearrangements, monogenic Mendelian disorders or poorly defined associations predispose to HSCR. In such cases, penetrance for the HSCR trait is of 5 to 70%, suggesting additional predisposing genetic factor(s). Bardet-Biedl syndrome (BBS) is a genetically heterogeneous multisystemic disorder characterized by postaxial polydactyly, progressive retinal dystrophy, obesity, hypogonadism, renal dysfunction, variable learning difficulties and, in about 5% of cases, HSCR. BBS proteins are involved in the assembly and function of primary cilia or the function of basal bodies, affecting both intraflagellar transport (IFT) and planar cell polarity (PCP).

RET is the major disease causing gene in isolated HSCR as well as a modifier gene for the enteric phenotype in some but not all HSCR-prone syndromes; CCHS, Down, and BBS are RET-dependent. Surprisingly, the greatest RET dependence was observed in BBS, although functional interactions between RET and BBS proteins are not known. We report the co-segregation of mutations at one BBS locus and at the RET locus for the HSCR trait to occur in BBS familial and sporadic cases. We show that human NCC possess a primary cilium and express BBS genes as well as many components of the PCP pathway. Genetic interactions between the BBS and RET pathways were supported by zebrafish double *Ret* and *Bbs* morphants in which enteric neurons were completely absent from the distal gut. These data emphasize the role of a primary cilium in NCC migration and enteric nervous system development.

#### C17.5

#### The common and specific genetic backgrounds of rheumatoid arthritis and celiac disease

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Recent genome-wide association studies (GWAS) discovered a number of genetic risk factors in autoimmune and inflammatory disorders and, strikingly, several of the associated genes and pathways are shared between various autoimmune diseases. Rheumatoid arthritis (RA) and celiac disease (CD) are two common, T-cell mediated, autoimmune disorders, which co-occur in families; both are strongly associated to HLA.

We aimed to perform a cross-study to investigate the effect of known CD risk factors in RA and known RA risk factors in CD.

We selected all loci so far detected for both RA and CD in genome-wide association studies and replication studies, with a p-value cut-off

of  $p < 5 \times 10^{-6}$ . We completed genotyping of 11 CD and 11 RA loci in 842 CD cases, 1370 RA cases and 1892 healthy controls from the Netherlands. We combined our results in a meta-analysis with earlier GWAS results from CD and RA in a UK population.

We confirmed association of the IL2/IL21 and TNFAIP3 loci to both diseases. We also found convincing association of the SH2B3 variant to both CD and RA, especially in an auto-antibody-positive subgroup of RA patients. Two more genes (LPP and PRKCQ) showed nominally significant association to both diseases, however, these findings need to be confirmed.

CD and RA share several risk variants for genetic susceptibility. A better understanding of the common and specific genetic profiles of autoimmune disorders would shed light on the disease pathology and could point to new drug targets for treating autoimmune diseases.

#### C17.6

#### Genome-wide association scan reveals major susceptibility locus for non-syndromic cleft lip with or without cleft palate on chromosome 8q24

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Non-syndromic orofacial clefts are considered to have a multifactorial etiology with a strong genetic background. The most frequent form is the non-syndromic cleft lip with or without cleft palate (NSCL/P).

We conducted a genome-wide association study involving 224 NSCL/P-patients and 383 controls of Central European origin in order to identify novel susceptibility loci for NSCL/P. A 640-kb region at chromosome 8q24.21 was found to contain multiple markers with strongly significant evidence for association with the cleft phenotype, including three markers which reached genome-wide significance. The 640-kb cleft-associated region was saturated with 146 SNP markers and analyzed in our entire NSCL/P sample of 462 unrelated patients and 954 controls. In the entire sample, the most significant SNP (rs987525) had a P value of  $3.34 \times 10^{-24}$ . The odds ratio was 2.57 (95% CI: 2.02-3.26) for the heterozygous genotype and 6.05 (95% CI: 3.88-9.43) for the homozygous genotype. The calculated population attributable risk for this marker is 0.41, suggesting that this study has identified a major susceptibility locus for NSCL/P. The locus is devoid of any known protein-coding genes. It is possible, that the observed association may mediate its effect by as yet unknown transcripts mapping within the region. An alternative explanation is that the disease-associated region contains cis- or transacting elements which control the expression of more distant genes. Interestingly, we found no evidence of any interaction between the 8q24.21 locus and *IRF6*, the only generally accepted NSCL/P gene to date. The study was supported by the DFG.

#### C18.1

#### Links between psychological characteristics and Distress after genetic result announcement : emotional regulation and adjustment

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Distress after genetic result announcement is at major stake for health practitioners. Researches tend to demonstrate that genetic result

leads to distress experience when linked with particular psychological characteristic (Smith, 2007) or coping strategies (Terciak, 2001). We propose here a biopsychosocial model highlighting the importance of emotional functioning prior to the genetic testing and emotional regulation process to explain psychological distress after genetic results. We have proposed questionnaires to 71 patients undergoing genetic testing for colonic cancer. Depressive symptoms (CES-D), State and Trait anxiety (STAI), coping style/coping strategies (WCC) and difficulties to identifying and verbalizing feelings (TAS20) were assessed before the genetic counselling and after results discovery. Results highlight that prior to genetic testing, state anxiety is best predicted by negative affectivity and a disposition to use emotional-focused coping to face with stressors. A greater state anxiety, social support seeking style and lesser problem-focused coping style prior to the test, associated with greater emotional coping strategies and higher difficulties to express feeling after the result, were best predictors of anxiety. Being mutation carrier does not show any effect on psychological distress. Implications to genetic counseling and care giving of subjects implied in a genetic testing diagnose are discussed.

#### C18.2 Evaluation of risk prediction updates from commercial genome-wide scans

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Commercial internet-based companies offer genome-wide scans to predict the risk of common diseases and personalize nutrition and lifestyle recommendations. These risk estimates are updated with every new gene discovery. In order to assess the benefits of updating risk information in commercial genome-wide scans, we compared type 2 diabetes risk predictions based on *TCF7L2* alone, 18 polymorphisms alone, and 18 polymorphisms plus age, sex and body mass index. Analyses were performed using data from the Rotterdam study, a prospective, population-based study among individuals aged 55 years and older. Data were available from 5297 participants. The actual prevalence of type 2 diabetes in the study population was 20%. Predicted risks were below average for carriers of the *TCF7L2* CC genotype (predicted risk 17.6%) and above average for the CT and TT genotypes (20.8% and 28.0%). Including data on the 18 polymorphisms caused 32% of participants to be reclassified (i.e. switched between below and above average): 25% of the CC carriers changed to increased risk, 46% and 4% of the CT and TT carriers changed to decreased risk. Including information on age, sex and body mass index caused 28% to change categories (27%, 32% and 18% for CC, CT and TT carriers respectively). In total, 39% of participants changed categories once when risk factors were updated, and 11% changed twice, i.e. back to their initial risk category. Updating risk factors may produce contradictory information about an individual's risk status over time, which is undesirable if lifestyle and nutritional recommendations vary accordingly.

#### C18.3 Compliance to screening and prevention guidelines of women carrying a BRCA1/2 mutation

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<sup>1</sup>Department of Genetic Oncology, IPC, Marseille, France, <sup>2</sup>Department of Surgery, IPC, Marseille, France, <sup>3</sup>Department of Radiology, IPC, Marseille, France. Clinical management of BRCA1/2 mutation carriers has recently been standardised. However, few data are available on actual risk management behaviours of mutation carriers. We reviewed medical files of mutation carriers in order to describe the use MRI screening, prophylactic mastectomy/oophorectomy, focusing on women tested after 2006 at the time follow-up was more standardised.

Between January 1998 and 2009, we identified in our center 295 families with a BRCA1/2 mutation comprising 214 women who tested positive and who were informed about their status and provided with management guidelines. Among them, 126 were identified after 2006, 104 were aged between aged 30-60 years old (mean age = 45.1; SD=7.8) of which 29 healthy carriers. Over the entire time period, among women free of ovarian cancer, 61% over 40 years old elected for prophylactic oophorectomy and 18.6% over 30 for prophylactic mastectomy.

Among women with a mastectomy for breast cancer, 37.7% elected for contralateral prophylactic mastectomy. In women tested after 2006, 58% (64% among women under 45 vs 51% among older women NS) had an adequate breast cancer risk management (screening comprising MRI or prophylactic mastectomy). Among the 38 women for whom management was not recorded as adequate, 4 were not eligible for MRI, 19 were followed outside our center and lost to follow-up and 11 had an advanced ovarian cancer.

These data provide preliminary information on the compliance to screening and prevention guidelines of BRCA1/2 mutation carriers. Rates of prophylactic oophorectomy, and adequate breast cancer risk management appear to be high.

#### C18.4

#### Community views of population carrier screening for fragile X syndrome

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Fragile X syndrome (FXS) is the leading cause of inherited intellectual disability. Currently, cascade testing of affected relatives fails to detect the majority of carriers, hence population carrier screening has the potential to identify more carriers, providing them with information about their health and risk of having a child with FXS. We explored community views of population carrier screening for FXS, using a qualitative approach.

Interviews and focus groups were conducted with 188 participants: healthcare providers (81), relatives of people with FXS (29), and the community, including pregnant and non-pregnant women (78).

Overall, participants supported population carrier screening for FXS, provided it was optional. Some participants did have concerns which were more focused on issues around screening in general and implications that screening has for society rather than about screening for FXS. Participants felt the ideal time to offer screening was before pregnancy and that screening should be offered through general practitioners clinics; however, some believed prenatal screening is more practical. Most felt that genetic counsellors should deliver the results and provide follow-up support. There was a sense of general lack of awareness about FXS, suggesting it is imperative that detailed information about the condition that people can relate to is provided at both pre-test counselling and when giving a positive result.

The results from this first large qualitative study exploring community views provide support for screening and are a valuable insight into the wide range of issues involved. These findings will help inform future development of carrier screening programs.

#### C18.5

#### Effect of Education, Knowledge and Experience on Acceptance of First Trimester Screening for Chromosomal Abnormalities

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First trimester screening by combined risk assessment for specific chromosomal abnormalities is accepted by the majority of women in Iceland. An underlying ethical principle is that involvement in screening programs should be voluntary.

Apart from information given by health professionals, pregnant women receive information from various sources such as friends, relatives, media and education. We investigated the effect of education, knowledge about screening and the direct experience of congenital disabilities on acceptance of prenatal screening, in five different maternity care clinics in Iceland. Self-completed questionnaire were completed by 379 participants. The age distribution, experience and education of participants were in accordance to the general population. Those with higher knowledge score were more likely to accept a screening offer than the least knowledgeable ( $p = 7 \cdot 10^{-6}$ ). There was a significant dif-

ference ( $p = 0.033$ ) in the likelihood of accepting screening between those with university or high-school education, 69% (n=264) compared with those in the group with elementary school or vocational education (n=94, 57%). Expectant mothers with experience of congenital anomalies in their own families were more likely to accept screening than those with no personal experience, 76% (n=79) versus 63% (n=188) of the others ( $p = 0.017$ ). The expectant mother's age had no effect on the acceptance likelihood. Many factors affect women's acceptance of first trimester screening including personal factors. Our results suggest that increased education, better general knowledge and former experience of congenital disabilities increase the acceptance rate for first trimester screening for chromosomal abnormalities.

#### C18.6

#### Genetic counselling and cardiological care in predictively tested hypertrophic cardiomyopathy mutation carriers: the patients' perspective

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Introduction: Hypertrophic cardiomyopathy (HCM) is an autosomal dominant heart disease associated with sudden cardiac death. Predictive genetic counselling and testing are performed using adapted Huntington guidelines, i.e. psychosocial care and time for reflection are not

obligatory and the test result can be disclosed by telephone or mail. Carriers identified by predictive DNA testing are advised to undergo regular cardiological follow-up to prevent sudden cardiac death.

Methods: We evaluated the opinion of 143 predictively tested HCM mutation carriers on received cardiogenetic care using questionnaires (response rate 86%). Predictive genetic counselling and DNA testing were evaluated on four domains: information provision, satisfaction with counselling, social pressure in DNA testing and regret of DNA testing. Opinions on cardiological follow-up were assessed pertaining to communication, nervous anticipation, reassurance and general disadvantages.

Results: Genetic counselling was valued positively. Only four carriers would rather not have known that they were a mutation carrier. A majority received their DNA test result by mail or telephone, and almost all were satisfied. Only 76% of carriers received regular cardiological follow-up. Those who did, had a positive attitude regarding the cardiological visits. General disadvantages of the visits were valued as low, especially by older carriers, men and carriers with manifest HCM.

Conclusions: We conclude that our adapted Huntington guidelines are well accepted and that cardiogenetic care is generally appreciated by predictively tested HCM mutation carriers. To better understand the cause of the substantial portion of carriers not receiving regular cardiological follow-up, although recommended in international guidelines, further research is needed.

## P01. Genetic counseling Genetics education, Genetic services, and Public policy

### P01.01

#### Association analysis of the ACTN3 R577X polymorphism and performance phenotypes in young soccer players in Slovakia

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Physical performance - related phenotypes are influenced by a combination of genetic and environmental factors. The functional allele (577R) of ACTN3 gene, which encodes human α-actinin-3, has been reported to be associated with elite sprint athletic status. The 577XX genotype enhances endurance performance. The aim of the presented study was to determine the frequency distribution of ACTN3 (R577X) gene in children various sports group from Slovakia and detect if genotype ACTN3 R577X polymorphism influences sprinting ability in an selected Slovak population. Genotypes were determined using Real Time High resolution melting PCR method. The results were compared with those 156 children various sports group and 150 sedentary controls. The % distribution of R and X alleles in children was significantly different from controls. In association part of study of 33 young soccer players in Presov, who were 12 years old we show that there is a significant association between the ACTN3 R577X polymorphism and 10m sprint time in males with the 577R allele contributing to faster times in an additive manner. The R577X polymorphism is not associated with other power phenotypes (30m sprint and agility run, the hurdle run, shuttle run test). Sports gene test can help predict talent from a very early age. Genetic screening is also being used to individualise training programs to suit a person's genotype.

### P01.02

#### Study of public opinion about some aspects on biobank creation and using

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The term "biobank" is not well known in the Russia, but there are many collections of biological samples in some research centers (Moscow, St.Peterburg, Tomsk, Novosibirsk, Ufa, Yakutsk). The availability of valuable biological samples and associated data, from one side, and approval of new ethic requirements for handling biological samples, from other side, suggest that establishment of biobanks on the basis of these collections is attractive. Biobanks can provide high quality of research and promote integration into research consortiums. To study public opinion about biobank in Tomsk, we have questioned about 800 Tomsk habitants (78% of women and 22% of men). 68,5% respondents were under 40 years. 67% of respondents had higher or specialized secondary education, 6% had science degree. 61% of habitants heard about biobank and its function, 84% of them note that creation of biobank in Tomsk is advisable. Only 56% agree with storage their biological samples in the biobank and using them in genetic studies. 59% are ready to provide additional information about their health and 79% want to know results of studies which use their samples. 59% respondents are afraid of information leak and 46% are afraid of discrimination by the results of their DNA study.

Thus, despite of some fear, most of respondents consider creation of Biobank in Tomsk as useful affair. There is need in additional public information about advantages of the biobank as well as development of strict ethic rules.

The study was supported by Russian Foundation for Humanitarian Research, grant No. 08-06-00514.

### P01.03

#### The role of religious involvement, knowledge about and attitudes towards adult genetic testing in formation of intentions-to-test - a structural equation modeling approach

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Purpose: To investigate the role of knowledge, religious involvement, previous experience with and attitudes towards genetic testing for adult-life disease susceptibility on the formation of intentions to undergo such testing.

Methods: Cross-sectional survey of a representative sample of 1,824 adults US adults ages 18 and over from the UNITED STATES PUBLIC KNOWLEDGE AND ATTITUDES ABOUT GENETIC TESTING, 2000. M-plus structural equation modelling (both confirmatory factor analysis and path analysis) of total, direct and indirect effects of knowledge, religious involvement, experience and attitudes on intention-to-test for adult disease susceptibility.

Results: Of the 1,824 adults surveyed, 77% expressed willingness-to-test for curable disorders, and only 52% for incurable disorders. 17% had some previous experience with a genetic disorder, and 8% had previous experience with genetic testing. SEM analysis revealed the following significant direct effects on intention to undergo testing: attitudes (positive effect; p=0.000), knowledge (negative effect; p=0.009), and previous experience (positive effect; p=0.034). Religious involvement showed a significant indirect effect on intention to undergo genetic testing, via a negative effect on attitudes (p=0.000); namely, individuals with high religious involvement were more likely to hold more negative attitudes towards genetic testing and to express less willingness to obtain genetic testing. The model as proposed explains 15.2% of the variance in the intention-to-test.

Conclusion: A majority of respondents indicate willingness to test for adult life genetic disorders, especially for curable disorders. Our findings underscore the need for refinement of outreach and intervention efforts, to account for multiple influences (knowledge, experience, attitudes and religiosity) on willingness-to-test.

### P01.04

#### Cross-cultural Communication in Genetic Services: Experiences in creating a network

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The two major communities (Greek and Turkish Cypriots) in Cyprus were separated by a border since 1974. The sharing of healthcare services, between both communities became very difficult. Recently efforts to bring the two communities together have been underway.

The importance of a multidisciplinary team with cultural competence is essential for the best care of patients with rare diseases such as genetic conditions. Good communication between health professionals, therapists and patients within such a team is fundamental. The multicultural composition of Cyprus created the need for a network to establish new and expand the existing channels of communication between health professionals. Patients/families were also invited to share problems, experiences and hopes while contributing in building a network for better communication and multidisciplinary care.

Attempt to create a network in the care and referral of patients, living with or at risk of genetic/inherited conditions, in both communities in Cyprus officially began in December 2006. At the end of 2 years, a network of communication of 181 people (including patients, physicians, speech therapists, scientists, psychologists, special needs specialists and counselling students from both communities) was created. A trilingual website and 6 trilingual leaflets on genetic conditions have been also been created as part of our public awareness campaign. Following a strenuous campaign, we were expecting a higher number of participants; however it was evident that cultural difference was a significant barrier to our efforts.

The poster will discuss the outputs of the two year project and the experiences of the project team.

**P01.05****The Department of Medical Genetics of MAPS: 20 years experience of teaching****M. O. Mkheidze;**

*Medical Academy for postgraduate studies, St.Petersburg, Russian Federation.* There is special structure of medical education to improve professional skill in Russia. It provides for periodical training at the institutes of advanced medical studies like St.Petersburg MAPS. Genetics and molecular medicine are the most important part of modern public health service. Over a long period of time in the USSR genetics and medical genetics were prohibited and a lot of physician generations were not able to study human genetics. Now it is impossible to be the successful physicians without knowledge of clinical genetics. The Department of medical genetics of MAPS was organized in 1989. Professor Svetlana Klueva (1931-2005) was the first head of the Department. There are two principal trends of the staff activity 1) training the physicians for practical genetic service and 2) teaching medical genetics for all doctors with basic clinical specializations. During 20 years 188 of training courses have been organized by the staff of the Department of Medical Genetics of MAPS in St. Petersburg and all over USSR and Russia. 6500 of physicians of the basic clinical specialties and geneticists have been studied. About 10000 of probands with hereditary and congenital diseases and their families have been examined. The principal goal of our staff activity consists in forming of genetic thinking in the physician community

**P01.06****Comparative policy analysis on direct-to-consumer genetic testing in South Korea and Japan****K. Muto<sup>1</sup>, H. Hong<sup>1</sup>, C. Chang<sup>1</sup>, M. Watanabe<sup>1</sup>, Y. Nakamura<sup>1</sup>, F. Takada<sup>2</sup>;**<sup>1</sup>*The University of Tokyo, Tokyo, Japan, <sup>2</sup>Kitasato University, Sagamihara, Japan.*

[Purpose] Direct-to-consumer genetic testing (DTCGT) refers to genetic tests that are marketed directly to consumers via television, print advertisements, or the Internet. As consumers can send their samples easily to neighbor countries, we need to share regulative policy options with other countries. Taking policy process approach, we explored and compared to clarify how regulative policies towards DTCGT have been determined in South Korea and Japan. [Method] Literature review and interviews to stakeholders [Results] In South Korea, Bioethics and Biosafety Act came into effective since 2005 whose provisions prohibit genetic testing outside medical institutions or without requested from medical professionals. The Ministry of Health and Welfare have responsibility to regulate genetic testing centers based on this act so that many DTCGT providers withdrew their business from South Korea. On the other hand in Japan, the genetic industry including DTCGT providers disclosed their original guidelines in 2008, supported by the Ministry of Economy, Trade and Industry. The Ministry of Health and Welfare take any actions towards DTCGT. Academia in human genetics has taken responsibilities in both countries. [Discussions] Totally different regulative actions towards DTCGT have been taken between South Korea and Japan because the initiative ministry is different. We need to start discussions with China, where DTCGT have close relationship with their national health care and R&D project, because most DTCGT providers from Europe and the States seek the new broad market just after clinical research in South Korea and Japan.

**P01.07****Reduction of the number of prenatal invasive techniques with the use of first-trimester combined screening for Down Syndrome****M. Perez Sanchez<sup>1</sup>, A. Mora<sup>1</sup>, P. Garrido-Fernandez<sup>2</sup>, A. Gonzalez<sup>2</sup>;**<sup>1</sup>*Hospital Virgen de las Nieves., Granada, Spain, <sup>2</sup>FIBAO. Hospital Torrecárdenas, Almeria, Spain, <sup>3</sup>FIBAO. Hospital Clínico San Cecilio, Granada, Spain.*

The first-trimester combined test (ultrasound measurement of nuchal translucency (NT), circulating levels of pregnancy-associated plasma protein-A (PAPP-A) and free beta-human chorionic gonadotrophin (b-hCG) has proved to be of value in prospective studies when screening for chromosomal defects, including those for Down Syndrome (DS), and can be a very good method for DS screening.

In our hospital has been implanted this method. The biochemical markers detection was carried out between weeks 9-13, and ultrasound measurement of NT between weeks 11-13.

From January of 2005, the screening was offered to all pregnant women of

our sanitary area and invasive techniques for prenatal cytogenetics diagnostic was indicated when a risk less of 1 in 300 for DS was obtained. The false-positive rate was of 3 % and with a detection rate up to 90 %.

The results show a high diminution (up to 55 %) in the number of invasive techniques from a median of 650 per year before first-trimester combined test implantation to a median of 265 after implantation. These diminution in the number of invasive techniques for prenatal cytogenetic diagnostic represent a less pregnancy lost due to invasive techniques with the same level of DS detection. Also will be of consideration the economic question, because a diminution in the number of invasive techniques also carries a decrease of the sanitary expense.

These results highly support that prenatal first-trimester combined screening for DS can be of election in order to diminish the pregnancy lost and the sanitary expense.

**P01.08****DYSCKERNE: Results from a pilot of the electronic Dysmorphology Diagnostic System (DDS)**

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The rarity of dysmorphic conditions means that even in Centres of Expertise, experience may be limited resulting in delayed or uncertain diagnosis. Correct diagnosis is the cornerstone of patient management, enabling clinicians to locate other patients with the same condition, share clinical experience and increase individual and collective knowledge about rare conditions.

The web-based electronic Dysmorphology Diagnostic System (DDS) developed by DYSCKERNE ([www.dysckerne.org](http://www.dysckerne.org)) links twenty existing European Centres of Expertise for Dysmorphology to form a powerful diagnostic resource for rare dysmorphic conditions.

Seventy-four submitting nodes are currently licensed to submit patients' clinical data and images to the DDS via an online case submission form. These cases are reviewed by the DDS Expert panel, comprising twenty-six Expert Dysmorphologists from twenty European Centres of Expertise, and a diagnostic report including suggested management plans for the patient is prepared from the consensus of expert opinions and sent to the submitting node.

Patient consent is an important part of the process, and Patient Information & Consent leaflets are available in a number of European languages. With appropriate consent, case histories are stored in an electronic archive which will be reviewed periodically and used to improve the definition and classification of syndromes, and facilitate further research into these rare conditions.

We present results from the DDS pilot, which has demonstrated that users have found the case submission system straightforward to access and easy to use. The Experts' discussion forums have facilitated lively debates about cases, and the number of suggested diagnoses received has been encouraging.

**P01.09****DYSCKERNE: Online educational tools for dysmorphology - examination of a fetus with congenital abnormalities**

**R. Day<sup>1</sup>, S. Gardner<sup>1</sup>, P. M. Griffiths<sup>1</sup>, C. Harrison<sup>1</sup>, K. Strong<sup>1</sup>, D. Donnai<sup>1</sup>, B. Kerr<sup>1</sup>, K. Metcalfe<sup>1</sup>, H. Brunner<sup>2</sup>, B. Dallapiccola<sup>3</sup>, K. Devriendt<sup>4</sup>, M. Krajewska-Walasek<sup>5</sup>, N. Philip<sup>6</sup>, J. Clayton-Smith<sup>1</sup>**

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External examination of a fetus with congenital abnormalities and/or dysmorphic features can be helpful in a number of ways. It can confirm or modify a suspected antenatal diagnosis, identify previously undetected problems, determine the underlying etiology of an abnormality or provide direction for further investigation. Such information can facilitate accurate genetic counselling to bereaved parents, especially

regarding future recurrence risks and options for prenatal diagnosis. Although there is literature outlining how useful external examination of a fetus with congenital abnormality can be, there is little available to describe how this should be done and what the examination findings may indicate. The aim of this tool is to provide such guidance in a modern, online format.

It has been developed within the DYSCERNE project, an EU funded project which has established a Europe-wide network of centres of expertise in dysmorphology. Further information about DYSCERNE can be found on the website [www.dyscerne.org](http://www.dyscerne.org) which serves as the access point to registered users for this educational tool.

The format is a short PowerPoint presentation which can be followed sequentially, or readers can navigate using links on the contents page to specific sections of interest. There are links to downloadable documents and useful proformas at designated points in the presentation which provide practical assistance with carrying out the fetal examination as well as detailed reference information.

This is the first in a series of tools created by the DYSCERNE project which aim to guide and educate clinicians throughout Europe on key aspects of clinical dysmorphology.

#### P01.10

##### DYSCERNE: Developing clinical management guidelines for dysmorphic conditions

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Delivering high quality genetic services requires healthcare professionals to develop evidence based guidelines which are subject to validation and quality checks. However, in clinical genetics, there is a paucity of guidelines, particularly for rarer conditions. Additionally, many guidelines produced have not used a robust methodology. A main aim of the DYSCERNE Network of Centres of Expertise in dysmorphology ([www.dyscerne.org](http://www.dyscerne.org)) is to develop management guidelines for dysmorphic conditions. A scoping exercise identified, Angelman, Noonan, Williams and Kabuki Syndromes as conditions that would benefit from guidelines. Guidelines will include; criteria for diagnosis, information on clinical management at different life stages, and when specialist referral is needed.

DYSCERNE's guideline development methodology utilises a modified SIGN (Scottish Intercollegiate Guidelines Network) methodology. SIGN's methodology involves systematic review and grading of published evidence, and uses multidisciplinary groups of clinicians to achieve expert consensus. This validated and internationally accepted methodology assumes a rich evidence base and has previously been used to develop guidelines for a range of conditions including cancers with strong genetic components. However, for rarer diseases, the evidence base is very small or non-existent, and we have adapted the process so more emphasis is placed on expert opinion and consensus, whilst maintaining systematic rigor and transparency.

Our programme of guideline development is ongoing and subject to continuous appraisal so modifications can be incorporated where necessary. The development protocol will be available on our website and it is hoped that others will be encouraged to utilise this approach to develop much needed guidelines for rare diseases.

#### P01.11

##### Role play as teaching tool for communication skills in prenatal genetics

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It is well known that role play is very effective learning tool to obtain communication skill. In Japan, our medical students learn communication and medical interview in 4<sup>th</sup> and 5<sup>th</sup> year of 6 years education.

In this study, we assessed the roleplays by 5<sup>th</sup> year student with the situation of prenatal settings to see how they could apply their skills to rather complicated cases. We made a lecture about prenatal tests and discussed with students beforehand and asked them to play as a

doctor, a nurse and a patient. This tutorial normally takes place for 2 or 3 students each time. We recorded all conversation with shorthand to look back easily just after they have done. We also discussed their skills and feelings afterwards. Role play time was about 35 minutes in average.

The result shows students are very flexible to put their knowledge into practical use in different situations and made supportive attitude. However it seemed difficult to choose less harmful words to explain. Interestingly, they pretty much included what they think interesting such as new genetics techniques rather than basic genetics to explain genetic conditions. In addition, it was easier than exam to see what and how they understand through observing how they explain each things and it was good for teaching staffs to review themselves. Roleplay in small group learning is good tool for teaching communication skills and difficult issues in clinical settings. It also was good opportunity to have active discussion in rather shy students.

#### P01.12

##### Nowgen's new Darwin-inspired molecular genetic workshop for school students

*L. E. Holmes<sup>1,2</sup>, K. Mathieson<sup>1,2</sup>, P. Finegold<sup>1,2</sup>, H. R. Middleton-Price<sup>1,2</sup>, D. Donnai<sup>1,2</sup>;*

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Nowgen works closely with students and teachers to ensure that the next generation appreciate the scientific, social and ethical issues associated with genetics. One part of this work is our programme for post-16 biology students. This year, to celebrate Charles Darwin's 200<sup>th</sup> birthday, Nowgen has been commissioned by the Wellcome Trust to develop and deliver a new molecular genetics practical workshop exploring human evolution.

This project has enabled Nowgen to push the boundaries of A-level practical work, serving as an exemplar to the scientific education community. The practical investigates the students' own genotypes at one variable SNP in the *TAS2R38* bitter taste receptor gene. Further bioinformatics work investigates the same gene in chimpanzees, to show that the two species have undergone convergent evolution, the acquisition of the same trait via independent biological processes.

During the one-day workshop students determine their bitter-tasting phenotype, extract their own DNA, set up PCR and restriction digest reactions, and run their results on an agarose gel. The results, viewed instantly with fluorescent DNA stain, provide information about their bitter-tasting genotype, leading to discussions regarding the correlation between phenotype and genotype and the level of determinism of our genes.

Our current A-level workshop is highly acclaimed by teachers, and heavily subscribed. Building on this success, Nowgen is delivering 75 Darwin-inspired workshops to 1875 students throughout 2009 - 2010. The practical will also be run in two other science centres in the UK, with a national impact on over 5000 students.

#### P01.13

##### Ethical issues and subsequent governance in the GEN2PHEN project

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The GEN2PHEN project aims to unify human and model organism genetic variation databases towards increasingly holistic views into Genotype-To-Phenotype (G2P) data, and to link this system into other biomedical knowledge sources via genome browser functionality. It involves participants from 14 countries. The regulatory and ethical framework was analysed for the 3 main levels addressed in the project: Getting data in; Data storage & infrastructure ; Getting data out. The work on ethical issues involved also examination by questionnaire plus interview of the ethical issues command by project participants, leading to the production of a grid of issues that should be applied to any set of data entering the G2P system or emerging from it. The main ethical issues examined were initial informed consent content in order to allow GEN2PHEN to be used, appropriate feedback to individuals and communities regarding results, anonymization check, manage-

ment of the right of individuals to withdraw data and/or samples from studies. The internal questionnaire allowed, after analysis of the 1st 20 answers to formulate a proposal of a governance system to prevent misuse of data and the protection of fundamental rights of participants in the research. Views were generally concordant on what ethical issues were, except on the level of individual information processed in the project, but various practical implementation of ethical principles were proposed, especially regarding the kind of ethics committee approval, the role of an internal ethics committee, the documentation of an appropriate consent and the use of ethnicity based information.

#### P01.14

##### A View of Ethical Issues in Medical Genetics in Iran

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Unexpected advances in medical genetics have provided the genetic testing tools for a number of genetic disorders which raises complex ethical, social, legal and religious issues.

Iran is an Islamic country with many different ethnic groups, high rate of consanguinity marriage resulting in high prevalence of recessive genetic disorders.

Bioethical decision making in Islam is done within a framework of values derived from revelations and tradition. A Fatwa (consensus edict), an authoritative ruling on a point of Islamic law, usually emerges in Islamic countries, consensus groups will typically include a broad and diverse representation of Ulema (Islamic jurists) and specialists, make a final decision about relevant subject.

According to a Fatwa in Iran the abortion is allowed after 120 days if there is a danger to maternal life, regardless of whether the fetus is normal or abnormal. In this case, termination of pregnancy goes against religious well-being, but it is done for the mother's physical health because protection of human life is one of the main principles of Islam.

Several strategic plans for medical ethics activities were performed by ministry of health and medical education.

Many ethical issues still need to be explored, although Islamic principles provide a moral and ethical basis for prevention and care in Muslim countries, there is much to learn from how other countries are challenging to do so within the context of their own moral and religious because a common rule in religions that is protection of humanity is the same.

#### P01.15

##### Change or not? Reflection on Eugenic ideas in the History of Human Genetics in Germany and Anglo-American Countries.

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Eugenics as a movement of large ambition had the idea of improving the genetic make-up of the human race in history. Since the era of racial hygiene in Third Reich the term has bad odour. In 1951 UNESCO said that there is one human race. Comparing the discussion about eugenics in Germany and Anglo-American countries there can be stated:

1. "Old Eugenics" before 1945 had social desire that was contrasting to "New Eugenics" with individual one. Before 1945 the decline of birth rate in upper class should be stopped. After then it became the wish for individual enhancement.

2. Eugenic ideas were topic in Germany and Anglo-American countries like in discussion at Londoner CIBA Symposium "Man and his Future" (1962) and following Marburger Workshop "Genetics and Society" (1969) and about those. Despite the past there was no difference in ideas and utopias.

3. Public policy on human genetics and reproductive medicine is more restrictive in Germany than in Anglo-American Countries. Although new ideas for perfection of man are discussed there is a big fear to get on a slippery slope. This influences the discussion about ethical aspects.

4. Historical experiences are always a strong argument in ethical and philosophical discussions. Lack of knowledge of the history of eugenics leads to arguments that are neither false nor true.

5. More and better information about eugenic history could help to develop public policy by preventing mistakes of the past. This may relax

research and therapy in human genetics and reproductive medicine.

#### P01.16

##### "Les couleurs de Jeanne" or "Jeanne's colours": a book to explain familial adenomatous polyposis to at risk children.

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Familial Adenomatous Polyposis (FAP) is an autosomic dominant predisposition to colorectal cancer due to mutations in the APC gene. Patients present with hundreds of colorectal adenomas which inevitably develop into carcinomas in the absence of early diagnosis and treatment using prophylactic colectomy. Children's patients are at 50% risk of receiving the mutation. Polyps can develop at very early ages. Usual recommendations are to start endoscopic surveillance from the early teens. In France, genetic testing of minors in FAP is usually offered after 10 years old. It is now well admitted that genetic testing of minors requires additional counselling considerations and effort to ensure understanding of the disease and of the gene test in order to reduce uncertainty and misperceptions.

Our experience of genetic testing for these children showed that discussions about the disease were often difficult between parents and their children. Informations about genetic transmission and risks of the disease are complex notions to explain to young children.

To improve communication within families and to help professionals dealing with genetic counselling, we created a book dedicated to these children. A professional drawer illustrated the story of Jeanne, a young girl, whose father has FAP and who will undergo genetic testing. Roche Diagnostics helped us to concretize this project by technical and financial support. This book has been sent to all French genetic centres dealing with this pathology and is available on request. We hope that it will help to improve management of these children and their families.

#### P01.17

##### Evaluation of a prototype assay suitable for Fragile X population screening

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Population screening has been proposed for Fragile X (FX) syndrome to identify pre-mutation carrier females and affected newborns. We have developed a prototype FX assay, which combines a single PCR using a "chimeric" primer with fragment analysis on the ABI PRISM® 3100 Genetic Analyzer (3100). In this research study, we evaluated the prototype assay performance using archived samples from routine clinical practice, representing a range of FMR1 CGG-repeat expansions.

205 samples previously genotyped at ARUP using a lab-developed PCR assay and Southern blot analysis were retested with the prototype FX assay. The prototype assay uses Celera's General Purpose Reagents along with a new primer set that distinguishes between normal and expanded FMR1 genotypes by PCR. Amplicons were subjected to fragment analysis on a 3100. Data were analyzed for the presence of a trinucleotide "ladder" extending beyond 55 repeats that was set as a cut-off to identify expanded FMR1 alleles. We identified the presence of expanded FMR1 alleles in 135 samples (63 pre-mutation, 69 full-mutation, 3 mosaics) and normal FMR1 alleles in 70 samples. We found 100 % concordance with previous results from PCR and Southern blot analyses. Individuals with expanded alleles can then be tested by diagnostic FX testing for allele sizing and methylation.

Using a single PCR combined with high-throughput fragment analysis on the 3100, we developed a rapid and reproducible prototype FX assay capable of distinguishing samples with expanded FMR1 alleles from samples with normal alleles.

#### P01.18

##### Genetic testing contribution to active longevity and life-span promotion

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The review of gene testing contribution into individual DNA data bank reflecting unique genetic peculiarities of each human, its major poten-

tial impact for achievements of active longevity and creation most favorable conditions for maximal duration of individual life-span. Participation of major age-regulated genes such as «biological clock» genes and the «weak chain» genes (predisposing to different multifactorial diseases) in ageing of humans is outlined. The significance of genetic testing of allelic polymorphisms and marker-genes implicated in common multifactor disorders such as bronchial asthma, osteoporosis, trombophilia, endometriosis, diabetes and preeclampsia is reviewed. Major problems evoked by sophisticated genetic testing results interpretation are considered. Special attention is paid to Genome Wide Association Studies (GWAS) technology implemented for analysis of genetic profiles and candidate genes associated with common diseases. Scientific problems and social interests in creation of individually oriented DNA-data banks (Gene Passes) amenable for the pregnant women, children, sportsmen, etc are discussed. The relationship of Gene Pass concept to the current international genetic program of «Personified Genome» is highlighted. Feasible perspectives genetic testing and basic contribution of Gene Pass into gerontology practical medical service are reviewed.

#### P01.19

##### Orphanet UK and Ireland, a growing Rare Disease resource

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Orphanet ([www.orpha.net](http://www.orpha.net)) the largest international online resource for rare diseases involves 38 countries and lists >11500 professionals. Orphanet features a comprehensive classification of rare diseases. Orphanet UK and Ireland was established in 2004 and has grown in depth, listing >2500 rare disease services and now featuring QA data and professional networks.

These cases show how patients, health professionals and researchers benefit from Orphanet.

Case 1: At 39 years Oliver started to bite his tongue without any apparent reason. He visited many professionals until in 2008 he was diagnosed with Neuroacanthocytosis. Through Orphanet he learned about his disease. He also found a support group for Neuroacanthocytosis patients and a specialised clinic close to home.

Case 2: Liz, a consultant specialising in lysosomal storage disorders, has a three month-old patient suffering from Gaucher disease type2. Through Orphanet she discovered a new orphan product based on a tartaric acid salt. She also learned about its potential benefits to treat this life-threatening disease due to its different mechanism of action in relation to other products.

Case 3: Tom is a researcher involved in a project about rare types of Arthrogryposis, including Kalyanaraman syndrome. Tom uses Orphanet to learn about these syndromes, find relevant articles and other European research projects. He uses its multiple disease classifications to discover links with other very different diseases having similar symptoms.

These cases highlight the importance of accessible and accurate information about rare diseases. They also illustrate the importance of a European approach to improve diagnosis, care and treatment.

#### P01.20

##### Unbalanced Offspring of Reciprocal Translocation Carriers: Genetic Counseling and Ethical Considerations

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Genetic counseling is the process during which information is given about a genetic condition. On the basis of two unbalanced offspring, ethical considerations and parental decisions during genetic counseling will be discussed.

The first family had a baby with multiple congenital abnormalities and a karyotype 46,XY,der(14)(1;14)(q42.1;q32.3)mat. Their major concern was the wellbeing of the child as the baby had growth retardation. The second family had a prenatally diagnosed fetus with a karyotype 46,XX,ish der(3)(3;13)(p22;q21)pat(wcp13+). The parents decided to continue the pregnancy.

In the first case, parents did not know about the consequences of an unbalanced child as they did not have the opportunity to have a genetic counseling session before the baby was born. They accepted

their child and were concerned about the prognosis of the baby. Second family had two counseling sessions, before and after diagnosis. In the first session, they were given information about the procedures to be performed, in the second; information about the unbalanced karyotype was given. They believed the child is healthy and stated they are ready for any difficulties to be encountered during lifetime of the child. Although, we offered termination of the pregnancy as an option, we admired their decision.

Genetic counseling sessions have three goals including; education and informing, providing support and help cope and facilitate informed decision making, need to be nondirective and patient relief is important. Social and educational facts as well as the practitioner's attitude play role in making decision, which sometimes has priority on the mentioned scientific facts.

#### P01.21

##### Role of the family in genetic counselling for predictive testing of late onset neurological diseases

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The role of the family in genetic counselling for predictive testing of late onset neurodegenerative diseases is a topic of growing interest and not yet sufficiently explored. Impact of involving the family in the dynamics of the predictive process remains quite unclear. The present longitudinal study aims at exploring the relationship between family and disease in the context of genetic counselling during pre-symptomatic testing (PST) for two neurodegenerative diseases: Spinocerebellar Ataxia Type 2 (SCA2) and Familial Amyloid Polyneuropathy ATTRV30M (FAP). For one year, we followed 35 subjects that carried out PST for SCA2 in Cuba and 28 subjects for FAP, in Portugal. It was shown that the family influences psychological behaviour of subjects requesting PST, starting from the decision making process down to the changed indicators of psychological wellbeing of those performing PST. After diagnosis, family functioning decreases regardless of the outcome, and the presence of the disease in the mother is associated with a greater disrupting effect of the disease in the family. In the future, additional short and long term longitudinal studies are essential, not just regarding the way in which the family influences genetic counselling process in predictive testing, but also on how the latter is influenced by the process of diagnosis.

#### P01.22

##### Process to incorporate genetic disease in the WHO international classification of diseases: tools managed by Orphanet

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The current International Classification of Diseases (ICD10) has undergone a revision process which should lead to a release of ICD11 in 2014. Most genetic diseases are absent in ICD10 and the ones having a specific code are often misclassified. As a consequence, morbidity and mortality due to genetic diseases is invisible in health information systems. To overcome this difficulty, Orphanet ([www.orpha.net](http://www.orpha.net)) has established a partnership with WHO to ensure a fair representation of rare diseases in general. In order to prepare the proposal, Orphanet has collected all published expert classifications and established a database of phenotypes indexed with ICD10 codes, MIM codes, genes, mode of inheritance, age of onset, class of prevalence. Phenotypes are assigned to as many classification systems as necessary to represent them. The visualisation of the classification systems and of the place of each disease within the classifications is available on the Orphanet website. A Topic Advisory Group on rare diseases has been established to manage the revision process at WHO. The whole community of experts is going to be involved in the validation of the proposals prepared by Orphanet during the year to come. Information will circulate via OrphaNews Europe. The human genetics community is invited to take an active part as the results will condition the visibility of all activities in the field.

**P01.23****Incorporating genetics into primary care: Education as a tool for health care professionals. The CAPABILITY ARGENTINA Demonstration Project**

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**Background:** We present the preliminary results of the Demonstration Project of CAPABILITY in Chaco a Northeastern Argentine province. Chaco has a highly endogamic population and no genetic services were available. CAPABILITY is a 3-year Specific Support Action for the Network of Excellence EuroGentest (2007-2009).

**Objectives:** The main objective of the Demonstration Project in Argentina is to enhance genetic services, using training of the health care team as a resource as well as to encourage the creation of complementary projects financed by Argentina to maintain the structure achieved and to reproduce the project in other areas of the country in correlation with Needs Assessment Argentina.

**Methods:** We have designed a logical framework for the planning of these processes, performed a diagnosis of the local situation, presented the project to the health authorities, set up a platform with teaching materials (PP presentation, printed syllabus, and CD) and a web page, chosen a zone for the pilot workshop and implemented it in April 2008.

**Outcome:** Through training primary health care professionals who receive patients with congenital defects have learned how to detect risk factors and recognize dysmorphisms. The number of interconsultations in the period April-December 2008 increased significantly compared to the period April-December 2007. This increase has led to a further need for genetic services and the building of a Genetic laboratory in Chaco has been planned. We have ensured that the project will be funded by the Hospital Garrahan of Argentina.

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**P01.24****The status of genetic information in health systems: a European view**

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Genetic information can be considered as sensitive personal information or health information and is regulated as such in many health systems. In a number of cases its production and uses are specifically regulated by law or other kind of regulatory texts. There is a huge variability in the kind of regulations that apply to genetic information in European countries and we analyse the relevance and influence of supra-national texts in such regulations. The most recently adopted text on this topic is at the Council of Europe level, namely, the "Additional Protocol to the Convention on Human Rights and Biomedicine, concerning Genetic Testing for Health Purposes" Strasbourg, 2008 (<http://conventions.coe.int/Treaty/en/Treaties/Html/203.htm>); it specifies a number of points complementary to those addressed in the "Convention on Human Rights and Biomedicine", 1997 (<http://conventions.coe.int/treaty/EN/Treaties/Html/164.htm>). We will analyse this document in relation to the situation in some chosen European countries (including France, Spain, Sweden), especially with regard to definitions used and principles applied, and with reference to the 25 recommendations published by the European Commission in 2004 (<http://ec.europa.eu/research/conferences/2004/genetic/>) following the work of an expert group on ethical, legal and social aspects of genetic testing. The focus will be on the issue of genetic exceptionalism, the place of genetic information in public health regulations and how direct to consumer offers on internet may challenge the existing regulations. This is of particular relevance as regard to the French situation where the law that regulates genetic testing is a "bioethics law" that is under revision in 2009.

**P01.25****A comparison study of the practices of genetic counsellors in EUROPE**

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Genetic counsellors and genetic nurses are health professionals with specialized training and experience in the areas of clinical genetics and counselling. They work as members of the multi-disciplinary healthcare team that provides genetic services.

In most European countries, the profession of genetic counsellor is relatively new and is not always recognized. The European Network of Genetic Nurses and Counsellors is working to establish standards of practice and education to support developments in the profession in Europe. The aim of this survey was to collect baseline data on practice and education of genetic counsellors in European countries.

Members of the Network were asked to respond to an electronic survey on the practice of genetic counselling in their own country. Data have now been collected from respondents from 13 countries.

This survey indicates that there are many differences in practice and training across Europe. There is no legal standing for the profession in some countries, so that genetic counselling could be practiced by members of other professions. The training may be provided in a hospital or university department of medicine, science or nursing. There are also marked differences in the types of clinical responsibilities undertaken. In some countries, the genetic counsellor can only undertake limited duties, such as pre-clinic contact, while in others he can work more autonomously, providing the full episode of care.

These data will help to provide a foundation for further development in this profession.

**P01.26****The impact of current motivational behaviours on the future of genetic medicine and research. A systematic literature review.**

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**Background:** Ambitious longitudinal studies, such as Biobank UK, rely on high volunteer recruitment rates. It is important for these studies, and for the subsequent application of genetic medicine, to understand motivational factors for the participants. Health behaviour models have been used to predict responses and explain attitudes about taking up healthcare.

**Method:** We carried out a systematic review that aimed to evaluate all current evidence for motivation to participate in genetic testing and summarize the findings using thematic analysis, grouping results based on health behaviours. We completed a search of qualitative and quantitative literature from 1996 to December 2008. Relevant studies were identified from searches of AMED, EMBASE, MEDLINE, PsycINFO and CINAHL databases. 342 relevant articles were retrieved in abstract form, of which 18 were found to match our inclusion criteria for review and data extraction.

**Results:** Analysis of the results encompassed multiple motivational factors for willingness to participate in genetic testing. Specific examples of grouped themes included:

- Personal, social and family history as predictive factors to participate.
- Perceived self-benefit from the development of knowledge and treatment of disease.
- Perceived social benefit, aiding the understanding of the wider community.
- Ability to understand terminology and education in genetic research as cues to action/participation.

There is a great need for the further development of our understanding of what motivates individuals to participate in genetic testing. Understanding these underlying motivators can facilitate education, health promotion and enhance both participation in future genetic studies and the application of new technologies.

**P01.27****Genetic screening programmes in Europe**

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Objectives: Genetic screening is defined as any kind of test performed systematically for the early detection or exclusion of a genetic disease, genetic predisposition or resistance to a disease, or to determine whether a person carries a gene variant, that may produce disease in his or her offspring.

Methods: This survey intends to present the current (2006-2008) status of genetic screening programmes as a best-effort collection of the genetic screening landscape in selected European countries, building on a first assessment of genetic screening programmes in Germany with the production of a final report in 2003. The data collection was performed on the basis of sources via internet, including PubMed search, websites of national screening authorities and societies and some other organisations, as well as data from a self-designed questionnaire, addressing the conditions screened in prenatal, population-based carrier, and cascade screening programmes, and organisational aspects of screening programmes in selected European countries.

Results and discussion: In almost all countries, there are nationwide newborn screening programmes for PKU (in Finland as regional programme targeted to immigrants) and for CH. Apart from Estonia, Finland, Latvia, Lithuania, Norway and Slovenia there are other conditions screened in different countries. These additional conditions vary per country, sometimes depending on prevalence but more often for historical and/or political reasons. A data comparison shows that there is a heterogeneity in conditions screened, screening methods, organisational aspects of screening programmes, and conditions screened in prenatal, population-based carrier, and cascade screening programmes among the European countries surveyed.

**P01.28****People's concerns and attitudes towards genetic testing for multifactorial diseases**

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Research Institute of Medical Genetics SB RAMS, Tomsk, Russian Federation. The high rate of genetic technologies developments and their forthcoming application into disease management and other spheres of life provoke a lot of concerns about its short- and long-term effects both in lay people and health care professionals.

A survey of 2000 Russian respondents had been conducted to study peoples' attitudes towards different aspects of genetic testing.

Results: 85% of respondents expressed their desire "to know more about genome"; 68% of survey participants would like to know about probable future diseases; and 89% believed they would try to change their lifestyle or take medications in order to avoid a disease if a high risk were identified. Among the reasons which can force people to undergo genetic testing the most important was people's anxiety about health which was related to the self-reported individuals' health status. 18% of respondents answered that "nothing" can prevent them from undergoing genetic testing for disease predisposition. At the same time 48% believed that if genetic testing results will be publicly available, tested people can be discriminated in society.

Results of the survey confirm the high level of public interest and a very positive perception of novel genomic technologies as well as highly overestimated expectations which call for special efforts in educating lay people and health care professionals. The high level of people's concerns about genetic discrimination argues for the necessity of regulation in this sphere.

**P01.29****EuroGenGuide: Patient Led Education and Development for Genetic Testing in Research and Medicine**

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Genetic Interest Group, London, United Kingdom.

EuroGenGuide is a EC-funded project, aiming to provide information about genetic testing and research to patients, the wider public and health professionals. The information will take the form of a 'manual' in two parts: one with information for patients or people considering taking part in research and the other with educational materials about genetics for health professionals.

There is considerable disparity in Europe between the relatively widespread availability of genetic tests and therapies, and the uneven distribution of information and access to both these and counselling services for those affected by genetic disorders. EuroGenGuide aims to reach into areas where information is limited or where clinicians and patients know little about genetics and the resources available to them in taking advantage of genetic technology and services, and help to resolve this often life-determining gap. EuroGenGuide's two-part manual will provide a readily-accessible comprehensive document and website for use by all which will help to generate a robust model of informed consent for those making decisions based on their options in respect of genetic testing or research.

The EuroGenGuide team is comprised of thirteen organisations from across Europe, and co-ordinated from London by the Genetic Interest Group. Now in its final year, the EuroGenGuide team is working to publicise and raise the profile of the guide ready for its launch in December 2009. Please feel free to get involved by registering to receive the newsletter, take the user survey and discuss issues of relevance on the forum, at <http://www.eurogenguide.eu>.

**P01.30****Genetics education for dental students**

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The aim of this study is to optimize the genetics education of dental students and residents based on the implications and applications of genetic knowledge and skills to dental health and patient care.

Materials and Methods: It was found a rich literature using PubMed search for articles on the topic studied. We used clinical cases in the collection of our genetics department too.

Results - The study of literature has enabled us evidence of many examples that demonstrate the use of genetic research in clinical practice: the identification of gene mutations that cause isolated inherited dental anomalies, gene mutations with pleiotropic effects, genetic testing for oral cancer susceptibility and pre-symptomatic diagnosis of periodontal disease. Changes of genetic structures produce the large diversity that may be encountered in clinical practice. In dental schools, educators and academic staff provide the opportunity for students to learn how to translate genes into dental health. Therefore, students and residents should be trained to understand the impact of genetic modifications on oral health, to recognize genetically determined or environmentally induced anomalies, to know the genetic tests available to identify the cause of diseases or abnormalities of craniofacial complex, to provide the patient best treatment possible based on the genetic profile and guide the patient to genetic counseling.

Conclusions - Genetics has the potential to shape the dental education and practice. In the context of genetic medicine, the dentist will integrate the tools of genetics in their dental practice for prediction, prevention and personalized therapy.

**P01.31****Genetics Made Easy - Non-profit informative web site on Human Genetics**

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EDUCATIONAL web site on human genetics.

GENETICS MADE EASY <http://www.geneticsmadeeasy.com>

Genetics made easy is a non-profit informative web site on Human Genetics that brings general population and scientific community closer together. The principal aim of the web site is to disseminate the scientific advances and knowledge that take place in genetics and how the general population (students, couples wanting to have a baby, clini-

cian and other healthcare professionals, law and legal field, journalism, etc.) can benefit from this progress.

The web is an **excellent complement to genetic counseling** and a **very useful teaching tool to clinicians** and other healthcare professionals, in order to complement medical personal consultations at specific points when teaching the patient, as you will see through the index web, as genetically inherited disorders are known across all medical specialties.

#### The index web is:

\* Introduction, \* The origin of life, \* Cell specialization, \* Chromosomes, \* How do we acquire our inheritance, \* What is heredity, \* Types of inheritance, \* Why do disorders develop, \* What happens when our recipes combine with our partner's recipes?, \* And how can we use this vast knowledge and benefit from it, \* Origin of hereditary disorders, \* Prenatal diagnosis techniques, \*Gene Therapy, \* Cloning, and stem cells, \*Questions, \* Links of interest, \* Further reading, \* Foreword.

A FORUM, is included.

Supplemented by static pictures and flash animation, at this moment is currently available on the Net in **Spanish, English, Chinese and Catalan**.

#### P01.32

##### Health care needs assessment for medical genetic services in middle- and low-income nations

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**Background:** Consequent on improving health, education and infrastructure many middle- and some low-income nations have experienced epidemiological transition resulting in congenital and common complex disorders becoming health care priorities. The WHO [2000] recommended that developing countries implementing medical genetic services should produce a 'country report', in essence a strategic health care plan. The first component of such a plan is a health care needs assessment (HCNA). In the 1970s industrialised nations standardised the process of HCNA. The approach was never applied to medical genetic services. Experience from advising middle-income nations on developing their nascent medical genetic services indicates they are not undertaking HCNA. A major impediment is the lack of a documented approach.

**Objectives:** To develop an evidence-based tool for HCNA for medical genetic services in middle- and low-income nations.

**Outcome:** CAPABILITY, an EU funded Specific Support Action investigating the transfer of medical genetic knowledge and technology to developing nations, has formalised a HCNA for medical genetic services that middle- and low-income nations can utilise for their 'country reports'. Key elements of the HNCA are the development of strategic aims; an evaluation of existing services and the environment in which they function; a review of epidemiology, effective interventions, opinions of consumers and professionals, available resources and possible constraints. Analysis of these enables the production of a HCNA. This approach will assist nations with emerging economies to marshal and allocate their limited resources when developing medical genetic services.

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#### P01.33

##### Huntington disease - concerns of family carers in the United Kingdom

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Huntington disease is a neurodegenerative condition. Affected individuals may require health and personal care for 15-20 years: this care is frequently undertaken by family members. Previous studies have demonstrated both the positive and negative impact of caring for relatives with debilitating conditions.

We report here findings of a study focussed on the concerns of family members caring for a person with Huntington disease in the United Kingdom. This was part of a larger study undertaken in the United Kingdom and the US. UK respondents (n=120) who were currently caring for a relative affected with Huntington disease were asked to self-complete the Huntington Disease Family Concerns and Strategies Survey to identify areas of concern and the extent to which specific issues bothered carers. Items in the scale were derived from a previous qualitative study of carers. A series of open questions required participants to respond in their own words: 81 respondents completed those sections. The qualitative data were analysed thematically, three main themes emerged. These were: i) the carer's health, ii) caring for the affected person in an appropriate way and iii) accessing social and health care services. Carers were concerned about their own health and ability to continue to care. Some health professionals were perceived to be uncaring and lacking knowledge to support care, while appropriate services were often difficult to access. Despite living in a country with a National Health Service, carers still have considerable concerns and stresses associated with support of a relative with a long-term genetic condition.

#### P01.34

##### Basic criterias of psycho-medical-genetic counseling of families with children affected by Down and X-fragile Syndromes in Republic of Moldova

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Psycho-medical-genetic assistance, expressed as counseling activity, is dealing with disorders of emotion, will, motor function and speech which are most frequent aspects of psycho-medical retardation of children affected by Down and X-fragile Syndromes, most frequent genetic pathologies in Moldova. Psychophysical retardation of children with genetic pathologies, in general, is not so evident in first stages of their life comparing with healthy children, due to human biological inborn potential. Developmental gap between healthy children and those affected, progressively and naturally increases later.

Correctional strategy of psycho-medical-genetic counseling of families for medical-psycho-behavioral stimulation of children up to three years old with Down and X-fragile Syndromes is based on following. First, amelioration of children's development by applying in complex of medical, psychological and pedagogical resources. Success of psycho-medical-genetic counseling of families with affected children depends on efficient applying of medical-genetic, psycho-pedagogical resources of amelioration of development of such children, especially in first three years of life. Second, earlier, differential stimulation of three basic psychic behaviors: psycho-motorial, social-affectional, cognitive-verbal. Evidently, earlier and individual applying of developmental-correctional medical, psychological and pedagogical resources, during counseling activity, determines successful rehabilitation and social integration of children, mostly in next periods of life. Third, support of children's families, which amplify the ameliorational effect of medical-genetic and psycho-pedagogical rehabilitational model promoted during psycho-medical-genetic counseling, based on partnership with mothers and/or other persons caring of these children, with the aim to contribute to stimulation of their development in families, representing most favorable ameliorational environment for children with Down and X-fragile Syndromes.

#### P01.35

##### Support for patients with multiple endocrine neoplasia type 1 and their families: what do they want?

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Multiple endocrine neoplasia type 1(MEN1) is a familial tumor syndrome which develops tumors in different organs and at different times, thus patients with MEN1 need to continue inspection and various treatment for the entire lifetime. Genetic testing of patients and family members can cause them significant psychological distress. To know what they have felt when they were diagnosed as having MEN1 and what kind of support they wanted, we carried out a written questionnaire-based survey for affected patients and their spouses. Twenty-nine patients and 22 their spouses completed questionnaires and those were analyzed.

More than three fourths and more than half of patients, respectively, were diagnosed based on results of genetic testing and family history. At the time of diagnosis, with surprise and uneasiness, many respondents concerned "inheritance of the disease to their children" as well as their own "future symptoms" and "future treatment". As time passes after the diagnosis, majority of patients reported a relief of anxiety, but some reported further increase of their anxiety, probably related to aggravation of the diseases and concern about genetic testing for their children. At the time of diagnosis, medical information was on the top of the list they wanted, but as time passed, they asked more social and physical support.

In addition to an appropriate medical management, sustained assistance which meets variety of needs of patients and the families is necessary to provide better quality of life to them, and establishment of an appropriate administration system is desired for that purpose.

#### P01.36

##### Molecular and cytogenetic laboratory of Leningrad province: 2008

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Molecular and cytogenetic laboratory is stationed at District Children Hospital. Last year it realized neonatal screening for PKU, CH, CF, galactosemia, cytogenetic investigations for making diagnosis of chromosomal pathology, prenatal screening for congenital defects (double-test), confirmation of hereditary diagnosis, medical care, long term inpatient and outpatient care, dietary management, genetic counseling. 10951 of newborns were examined through neonatal screening. Four children with PKU, two cases of CH, one case of galactosemia and one case of CF were diagnosed. Cohort of children with PKU, CH, CF and galactosemia has special dietary and medicinal treatment. Prenatal biochemical screening of the first ( PAPP-A, HCGb) and the second (alfa-FP, HCG) trimesters was performed for pregnant women (5962). Owing to realization invasive prenatal investigation (369) we were able to detect prenatally trisomy 21 (3 cases), Turner syndrome (2 cases), syndrome of partial deletion of chromosome 18 (1 case). We have detected some new cases of chromosomal pathology postnatally (in total 969 samples): trisomy 21 (22 cases), trisomy 18 (2 cases); syndrome of partial deletion of chromosome 5 (1 case), and some other cases with chromosome pathology. Five children with CF were found using sweat test for 260 samples. We continue to create register of inherited and congenital disorders among Leningrad province population.

#### P01.37

##### First experiences with the extended newborn screening program in The Netherlands

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By January 1<sup>st</sup>, 2007 the newborn screening program in the Netherlands was extended from three disorders to 17, including medium-chain acyl-CoA dehydrogenase deficiency and sickle-cell disease. The selection of disorders to be screened for was based on a report of the Dutch Health Council. Three categories were distinguished: disorders for which considerable irreparable damage can be prevented (category 1), disorders for which this applies to a lesser degree or for which the evidence is inconclusive (category 2), and disorders for which newborn screening does not prevent damage to health (category 3).

In 2007 a total of 194 infants were diagnosed in the neonatal screening program, including 60 with sickle-cell disease or a serious hemoglobinopathy, 70 with a metabolic disorder, 57 with congenital hypothyroidism and 7 with congenital adrenal hyperplasia. The uptake remained stable at 99.75%. Information in the third trimester of pregnancy was given to enable parents to make an informed decision on participation to the newborn screening and to decide on unintentionally found carrier information. The decision to participate to the newborn screening was an informed decision in 80% of cases. The decision (not) to opt out to receive information on carrier status was an informed decision in 60% of cases. The attitude to the newborn screening program is very positive, but information can be improved. Carrier status was often communicated without stressing the consequences of this information for reproductive choices of the parents. The next disease to be in-

cluded might be cystic fibrosis.

#### P01.38

##### Genetic counseling of phenylketonuria (PKU) patient's parents

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The PKU patient's parents appraisal of different aspects of genetic services in Russia in connection with neonatal screening was estimated. 261 respondents from 5 regions of Russia were participated in the research. Only 13.4% of respondents knew about the program of neonatal screening for PKU in Russia before the birth of the PKU child while over 85 % of them knew nothing about it. Many respondents specified the absence of the adequate information about screening for hereditary diseases in their comments to the questionnaire. Almost all respondents (97%) have received information about the hereditary character of their child disease from the genetic counselors, and 95.8 % of them have specified that they have understood this information. However only 62.9% of respondents could correctly specify the value of recurrence risk of PKU, and only 36.4 % of them could correctly attribute a risk category. From our point of view the absence of adequate information about neonatal screening as well as simultaneous providing information on hereditary character, recurrence risk and prenatal diagnostics of the PKU make genetic counseling of such families non-effective. Changes in methodology of genetic counseling of PKU families should be introduce in Russia. Prenatal diagnostics of PKU was acceptable for approximately to 40% of respondents, and the abortion of a PKU foetus is morally acceptable to about two-thirds of them.

#### P01.39

##### Correlation of clinic, genetic and epigenetic aspects implicated in the etiology of Prader Willi/Angelman syndromes: model of multidisciplinary approach for rare diseases in Romania

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The purpose of this research is to establish the new hypotheses that are responsible for Prader- Willi/Angelman syndromes. Our study has as objectives the implementation of new molecular methods for genetic/epigenetic investigation and establishment of national centers with high expertise in approaching the two syndromes, the rare genetic diseases that will develop educational reference and release centers. We also aim to evolve efficient partnership with patients associations through specific modalities like dialogue. The power of these associations will propel the research, will inform the patients and will respond to civil society questions. The study will establish international collaboration and partnerships with researchers having similar scientific interest, establish partnerships with PWS-Organizations, IPSWO, research groups from each country aiming financial support on programs that intend to stimulate collaboration between specialists, researchers and nongovernmental organizations. Finally, we aim to develop a multidisciplinary partnership, to build a common platform of activities for new innovative solutions in respect to rare disease needs. These new bridges of real and effective collaboration will ascertain on the national level the setting up of a solid network comprising institutions with high expertise in this domain, well connected to other national or international research networks. The results of the research will be published in well-known journals with high impact factors for enlargement of Romanian research visibility in international domain of rare diseases. In conclusion our project aims to implement in Romania the European model of the network for rare diseases research, model adapted with success in management of these diseases.

**P01.40****The role of Genetic counseling in Premarital screening program in Saudi Arabia.**

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Haemoglobinopathies are the most inherited disorders worldwide including Saudi Arabia which can be preventable with application of screening programmes. Ministry of Health in Saudi Arabia had initiated premarital screening program in all country regions. The aim of this study was to explore the impact of the PMS and genetic counseling on couples at risk for thalassaemia and sickle cell anemia in an area of the country with high hemoglobinopathy prevalence. A total of 129 candidates at risk were included, 98% of them proceeded with marriage. Culture pressure was the main reason in more than 48%. Although most of the candidates did not receive genetic counseling yet, the concept of genetic counseling was liked by most of them and the program helped in early detection of the disease in their offspring.

**P01.41****Towards implementation of non-invasive prenatal diagnosis in the UK National Health Service**

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Emerging technologies that capitalize on the presence of cell-free fetal nucleic acids in the maternal bloodstream have the potential to transform prenatal diagnosis and care by allowing non-invasive testing from as early as 7 weeks. This represents a major advance, since diagnosis currently requires a later, invasive procedure (amniocentesis or chorionic villus sampling) with an associated miscarriage risk of around 1%. Many women opt against prenatal testing for this reason, and hundreds of healthy fetuses are lost following testing each year in the UK alone. Technological applications already in active development are the determination of fetal sex or the diagnosis of specific diseases in the fetus of women at high risk of inherited disease; the determination of fetal Rhesus blood-group status in Rhesus-negative women at high risk of complications of pregnancy with a Rhesus-positive fetus; and the diagnosis of aneuploidies such as Down Syndrome in the fetus. The approach may permit diagnosis of a wider range of genetic diseases in the future, subject to certain limitations, and ultimately could also allow improved monitoring for complications of pregnancy such as pre-eclampsia. The PHG Foundation has led an expert working group of key UK stakeholders, including National Health Service (NHS) researchers, clinicians, managers and patient representatives, in an examination of current evidence and associated ethical, legal and social issues in this area. Findings and strategic recommendations for the effective and responsible implementation of this new technique into health services in the UK will be presented.

**P01.42****Public trust in media and science outreach on genomic studies: a comparative study between the public and scientists**

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Objectives: We've been conducting questionnaire surveys since 2005 to explore attitude change on genomic studies of the general public in Japan. To clarify their attitudinal change from science outreach and media interaction, we compare the two dataset from the general public (2005 and 2008) and the dataset from Japanese scientists (2007).

Methods: Self-reporting questionnaires were sent regarding molecular biology and human genetics/ genomics to the public (2005 and 2008) and scientists (2007) in Japan. They were asked about their value and risk cognition towards genome sciences, science outreach and media interaction. We compared these results with the dataset from 2,171 citizens in 2005 (RR=54.3%), the dataset from 1,613 citizens in 2008 (RR=53.8%), and the dataset from scientists in 2007 (RR=35.1%).

Results: Regarding media interaction, received media on genomic studies (multiple choices) in 2005 are "TV/Radio" (56.7%), followed by "Newspapers" (46.3%) and "None" (22.8%). This trend hasn't changed

in the dataset 2008. "Internet (3%) and "Lecture series (1.1%) are less requested media from the public. However, scientists responded that they wanted to provide their research results via "Lecture series (44.3%)", "Newspapers (43.4%)", "Internet (43.2%)". "Information on the Internet" is less trusted media in the public (11.5%) rather than scientists' forecast (33.0%). "Information from non-profit organizations" are least trusted (7-8%) from both the public and scientists.

Discussions: Citizen journalism plays important roles for science communication in Europe or South Korea, but not in Japan. We discuss this results with our coming final survey.

**P01.43****Effective strategies to generate public interest and dialogue in genetics**

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Public engagement is central to Nowgen's work, as we believe that the public need to be involved in shaping the future of genetic medicine. We aim to empower citizens to make informed decisions about new genetic technologies and health services. Nowgen is highly regarded in its work related to education and dialogue and is becoming increasingly involved in influencing policy at local and national level.

Nowgen has developed a comprehensive public engagement programme that incorporates a broad range of approaches, including: debates, exhibitions, working with artists, filming with teenagers, population surveys and patient engagement. Our most successful strategy has been to combine the display of visually engaging materials in the community, with events involving leading academics. This approach has stimulated public interest, generated significant media coverage and provided opportunities for the public to find out more from experts and discuss their views on scientific issues.

Two recent projects led by Nowgen have exemplified this approach. 'Faces of Manchester' involved the production of artwork about the human face, alongside events exploring human identity and genetics. 'Our Kid' led to the development of a multimedia sculpture displaying over thirty short films, alongside events highlighting biomedical research.

An independent evaluator has reported on the impact on these projects, finding that overall the audience response was very positive. The main strengths of the projects were to work with numerous community partners and use innovative, creative approaches to generate public interest.

**P01.44****Belgian strategies and actions for rare diseases**

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All rare diseases (prevalence of less than 1/2000) combined affect around 8 % of the population and are a public health priority of the European Union since 1999. A community action program (1295/99/EC) of the European Commission asked all member states to adopt a "national plan" on rare diseases by 2011. Belgium, internationally respected for its medical infrastructure, services and research, can also improve its suboptimal health care framework to address rare diseases.

The Belgian taskforce on rare diseases and orphan drugs, supported by the King Baudouin Foundation since 2006, is composed of a multidisciplinary team of stakeholders. Representatives of a.o. patient organisations, public health services and institutes, research centres and industry have successfully campaigned for a comprehensive Belgian national plan, in line with the decision of the European Union.

A strategic approach to improve the situation regarding diagnosis and treatment as well as the stimulation of rare disease research in Belgium will require many aspects to be considered. Already in place are e.g. reference centres for certain rare diseases, a framework for the approval and follow-up of orphan drug reimbursement and the Orphanet Belgium database. The latter is an important resource to build up for both patients and physicians.

The existing situation with regard to rare diseases in Belgium together with future plans as well as the progress of the Orphanet Belgium database will be presented.

**P01.45****Rhombencephalosynapsis and related anomalies: the largest fetal series lead to suggest a European network.**

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Rhombencephalosynapsis (RS) is a rare cerebellar malformation defined by vermian agenesis with fusion of the hemispheres and the dentate nuclei. Embryologic and genetic mechanisms are still unknown and to date, no animal models are available.

We have created a RS database to carefully review the phenotype observed in France including familial, clinical, radiological and pathological patterns. The morphological analysis of 40 foetuses (Pasquier et al., 2009, Acta Neuropathol 117: 185-200) allowed us to confirm that RS is always associated with other brain abnormalities and can be classified into three groups : 1- pure neurologic forms from isolated RS with hydrocephalus to supratentorial midline abnormalities (neural tube defect or holoprosencephaly), 2- syndromic forms with VACTERL-H association, 3- other syndromic conditions (Gomez-Lopez-Hernandez syndrome).

We have also collected several postnatal cases without hydrocephalus or severe neurological symptoms. This might suggest that RS is underdiagnosed during prenatal or postnatal periods (less than 80 cases reported in the literature). In order to precise its frequency, neurological prognosis and genetic counselling, we intend to create a European network to gather most of the rhombencephalosynapsis cases and share the results provided.

From DNA samples collected, this survey would also aim at looking for genetic factors using a pangenomic screening tool (CGH-array). This approach has already enabled us to identify 2 different micro-arrangements from a series of 20 cases. Contact: laurent.pasquier@chu-rennes.fr

**P01.46****Should we screen for gene mutations in blood samples with MCH greater than 27pg in areas with a high prevalence of thalassaemia?**

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Screening strategies for thalassaemia carriers are usually based on cut off values for red blood cell indices (either MCV or MCH). There is evidence for a higher sensitivity for MCH <27pg a criterion for  $\beta$  thalassaemia Screening in pregnant women. In this study, investigate single globin gene mutations in the normal populations with MCHs >27 pg.

Blood samples from 36 individuals with MCH greater than 27pg (MCV and Hb A<sub>2</sub> is normal too) were screened for presence of globin gene mutations and  $\alpha$  globin gene deletions by ARMS PCR, Sequencing and Multiplex gap PCR.

Twelve individuals harbored globin gene mutations. Of these, 3 had HbS, 2 had HbD, 2 had Cd75 mutations and 2 had deletions of one  $\alpha$  globin gene. Three individuals have  $\beta$  thalassaemia major; that we believe because of transfusion these indices is normal.

If one partner is found to be a carrier of a  $\beta$  thalassaemia mutation or single  $\alpha$  gene deletion, it is advisable to look for HbS or  $\alpha$  globin gene deletions in the other partner, even if MCH and MCV is within normal range.

**P01.47****How do neurologists explain genetic causes of sporadic adult-onset ataxia to patients?**

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The etiology of sporadic adult-onset ataxia is poorly understood, but Abele et al. reported that 13% of patients with negative family history have causative genetic mutations. We found two patients with SCA3 mutation, who had a history of a family member(s) having been diagnosed with sporadic ataxia several years earlier.

We studied how Japanese neurologists explained the genetic causes of sporadic ataxia to patients by referring to the clinical records. At the neurology clinic of Tokai University Hospital, 109 patients have been diagnosed with spinocerebellar degeneration (SCD) since 1990. Among them, 37 patients (34%) had positive family histories. Among the 72 patients with sporadic ataxia only 16 patients had received a clear explanation of the genetic causes from doctors, and 8 patients had received genetic tests (SCA1, 2, 3, 6, 17, DRPLA). Although no genetic mutations were found among sporadic ataxic patients who took these tests, mutations might have been present among those who did not take the tests. Family history may be negative because of early death or late onset of parents, failure to diagnose SCD patients among family members, reduced penetrance of the mutant allele, or a *de novo* mutation for autosomal dominant ataxic gene. Neurologists should consider possible genetic causes of sporadic ataxia.

**P01.48****Challenges in guiding first year medical students in a one semester genetic journey**

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Teaching is considered a well defined activity, which helped us all achieve our knowledge, as also did our predecessors, and as, we assume, will have to do our followers. Changes in everyday life, technology development, but also changes in human mentality and/or view of life, modified expectations in students as well as in teachers.

The dynamics of the teaching process is still under study and consensus has not yet been reached on exactly what teaching techniques should be used, to deliver the information and help students reach knowledge on specialty aspects that might suit their own future medical practice. Genetics is studied only in the first year at the Medical and Pharmacy University "Carol Davila", generating questions on the level at which it should be taught.

Different questionnaires about the practicals were answered by 90 Romanian and 38 foreign students. The last group was again divided because 15 students started later, had two practicals/ week instead of the usual one, and had only one assistant lecturer, who also evaluated them at the end of the practicals. Also 38 foreign students, some of which were also in the groups which received questionnaires about the practicals, were asked about the quality of 6 from 16 lectures.

After analyzing the results one can conclude the necessity of a better connection between lectures and practicals, the latter having to loose the mostly theoretical topics, genetics being a proper field for applying different teaching methods even if students have various biology background knowledge from high-school.

**P01.49****Development of timely and relevant training courses for healthcare professionals and genetic researchers**

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Techniques for analysing genetic variants related to disease and treatment are constantly advancing, and allow ever more sophisticated and rapid analysis of patient samples. The consequence of greater access to these techniques is that many clinical and laboratory disciplines have to embrace the new technology.

Nowgen, A Centre for Genetics in Healthcare, in collaboration with leading research and clinical scientists, has developed a portfolio of accredited training courses for genetics professionals involved in research and/or in the provision of clinical services. These courses have been developed following a thorough consultation with senior clinical

and research scientists to assess training needs. Furthermore, we have also developed courses in biobanking which are emerging as important resources for translating knowledge of the human genome into real benefits for health.

Courses launched in 2007 for clinical cytogeneticists and genetic counsellors have become well-established, receiving excellent appraisal from both delegate and professional bodies alike. The reputation of our courses in bioinformatics for cytogeneticists and molecular geneticists has led to them being repeatedly over-subscribed. More recent courses in Real-Time PCR and biobanking have attracted delegates from around the world and in 2009 we are launching courses in micro RNA analysis and array CGH.

To date, over 400 genetics professionals have attended our courses. These are part of a broader training programme at Nowgen that has attracted over 1,500 delegates in the past 5 years. We will continue to monitor emerging training needs of research and clinical scientists, to update existing courses and to develop fresh initiatives.

## P02. Clinical genetics and Dysmorphology

### P02.001

#### 3-M syndrome: A report of three Egyptian cases

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The 3-M syndrome is a rare autosomal recessive disorder. It is characterized by prenatal and postnatal growth retardation associated with characteristic features. We report on three patients from two unrelated families, including two male sibs, with the characteristic features and radiological findings of the 3-M syndrome. The main features in our cases were low birth weight, short stature, malar hypoplasia, prominent premaxilla, anteverted nostrils with a fleshy nasal tip, long philtrum, thick patulous lips, high arched palate, pointed full chin, short broad neck, broad chest with transverse grooves of anterior thorax, hyperlordosis, brachydactyly of hands and feet and prominent heels. Radiographic studies showed slender long bones and ribs, a narrow pelvis and foreshortened vertebral bodies. Our reported cases are the offspring of healthy consanguineous parents confirming the autosomal recessive pattern of inheritance in the syndrome. These are the first reported Egyptian patients with this rare disorder.

### P02.002

#### Parental origin and distinct mechanism of formation of the 48,XXYY karyotype, molecular characterization

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Among sex chromosomal aneuploidies, the addition of more than one extra X and/or Y chromosome are less frequent. We report a child with karyotype 48,XXYY. Molecular analyses are essential in order to determine the parental origin and mode of formation of the additional chromosomes. Quantitative fluorescent PCR (QF-PCR) was used including the amplification of amelogenin, which is present on both sex chromosomes in a biallelic form, SRY gene, a polymorphic short tandem repeat (STR) on the pseudoautosomal region of X and Y (X22), five polymorphic X-specific STRs, and a Y-specific marker (G10\_STS47). Molecular investigations were compatible with the described 48,XXYY karyotype. Ratio 2:1 corresponding to the peaks in the detected fluorescence signal of the X22 marker revealed the existence of the 47,XYY syndrome in the father (Karyotype pending). Molecular investigations of X STR markers indicated paternal origin of the additional X chromosome and, consequently an error in paternal meiosis I.

The QF-PCR technique resulted extremely sensitive to detect X chromosome anomalies also in postnatal diagnosis and evidenced an infrequent parental/meiotic origin of the 48,XXYY syndrome. Additional evidence came from molecular data and gained increased importance in this variant of Klinefelter's syndrome, in which distinct patterns of X-inactivation could play a role in the observed differences in the degree of clinical manifestations of patients.

Molecular and cytogenetic characterization	PATIENT	FATHER	MOTHER
KARYOTYPE	48,XXYY	XYY (QF-PCR)	XX (QF-PCR)
AMEL (Xp22.31-Xp22.1/Yp11.2)	XY	XY	X
SRY (Yp11.31)	PRESENT	PRESENT	NO
X22 (Xq28Yq)	199/225/241pb(2:1:1)	199/225pb(2:1)	220/241pb
DXS6854 (Xq26.1)	110/110pb	110pb	106/110pb
XHPRT (X26.1)	274/285pb	285pb	274/281pb
DXS6803 (Xq21.31)	110/120pb	110pb	120/120pb
DXS8377 (Xq28)	250/262pb	250pb	253/262pb
DXS6809 (Xq21.33)	263/271pb	263pb	267/271pb
G10_STS47 (Yq11.222)	PRESENT	PRESENT	NO

### P02.003

#### A review of congenital abdominal wall defects

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Aim: The pathology of umbilical region- middle celosomies have different embryology, clinical aspects, treatment and outcomes. Middle celosomies still represent a significant cause of morbidity and mortality in neonates.

Material and methods: This is a 9 years period retrospective review of patients whom were presented to the Pediatric Surgery Department with gastroschisis, omphalocele and umbilical hernia. Patient's personal data, positive diagnosis, presence of other congenital malformations and outcomes were recorded.

Main results: There were 72 patients (12 presented omphalocele, 24 gastroschisis and 36 umbilical hernia). The omphalocele and gastroschisis were more often observed to boys (58.33%) and umbilical hernia was more to girls (61.11%). Omphalocele type I Aitken occurred to 9 cases, type II to 3 cases, embrionar gastroschisis occurred to 15 patients and fetal gastroschisis to 9 cases. Other congenital malformations ( digestive- 12, cardiac and vascular anomalies-7, genitor-urinary-2, lungs-1 and locomotors anomalies-8) were presented in omphalocele and gastroschisis and no one in umbilical hernia. Pre-term neonates were more often in gastroschisis (18 cases -81.82%) then to gastroschisis (4 cases -18.18%). Surgical treatment was applied in all cases. Mortality was 16 percents in gastroschisis and zero in omphalocele and umbilical hernia.

Conclusions: Middle celosomies have requested surgical treatment. Outcomes are different according to stage of illness, gestational age and other anomalies associated. Total parenteral nutrition in postoperative period in gastroschisis and omphalocele increased the prognostic.

### P02.004

#### Array-CGH analysis in a patient with Acrocallosal Syndrome

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We present a patient aged 3 years with a clinical diagnosis of acrocallosal syndrome (ACS) and a complex chromosomal rearrangement (CCR). Pregnancy was unremarkable, except for the detection of corpus callosum agenesis in the second trimester. At birth, weight, length, OFC and Apgar scores were all within normal limits. Severe hypotonia, hypertelorism, strabismus and nystagmus, short philtrum, micrognathia, broad thumbs and toes associated with absence of intermediate and distal phalanges of fifth finger, talipes varus and micropenis were noted. Abdominal ultrasound examination detected bilateral kidney malrotation and bilateral vescico-ureteral reflux. Agenesis of corpus callosum was subsequently confirmed by cerebral MRI. Karyotype was 46,XY; Prader-Willi syndrome and mitochondrial disorders were ruled out. A complex chromosomal rearrangement consisting with a deletion (12)(p12.2p2.1) and a duplication (16)(q23.3) was detected by a-CGH analysis. Interestingly, the deletion and the duplication have been inherited from the phenotypically normal mother and father respectively and they are not described as polymorphic variants. The CCR detected in our patient has never been associated with ACS, but being inherited by phenotypically normal parents, its pathogenetic role is not clear and it is open to speculation.

**P02.005****Complex chromosomal rearrangement in chromosome 4q causes acrofacial dysostosis and cardiovascular malformation**K. M. Roetzer<sup>1</sup>, A. C. Obenauf<sup>1</sup>, K. Schoner<sup>2</sup>, M. R. Speicher<sup>1</sup>, H. Rehder<sup>3</sup>;<sup>1</sup>Institute of Human Genetics, Medical University of Graz, Graz, Austria, <sup>2</sup>Institute of Pathology, University Hospital Giessen and Marburg, Marburg, Germany, <sup>3</sup>Department of Medical Genetics, Medical University of Vienna, Vienna, Austria.

Acrofacial dysostoses (AFD) are characterized by the association of mandibulofacial dysostosis in combination with limb defects. It is a heterogeneous group of syndromes which contains many different entities, such as the Nager Syndrome, the postaxial (POAD) and the Rodriguez type of AFD. We describe a female fetus exhibiting many features of acrofacial dysostosis, including severe retro- and micrognathia, clinodactyly, arrhinencephaly and a complex heart defect. According to these findings, AFD Rodriguez type was suspected. This is a very rare disorder of unknown etiology first described by Rodriguez in 1990. Chromosome analysis had been previously performed in an outside lab and was reported to be normal (46,XX). Because of the complex heart defect, classified as an early absent pulmonary valve syndrome, fluorescence in-situ hybridization (FISH) for the 22q12 microdeletion region was performed. However, no abnormalities were found. Using high resolution array comparative genome hybridization (aCGH) a complex rearrangement on the long arm of chromosome 4 consisting of two large duplications separated by two microdeletions was detected. The involved region contains a large number of genes. Interestingly, one of the deleted genes encodes a transcription factor regulating the development of cardiovascular structures, especially of the right ventricle. Furthermore, it was previously shown in a mouse model that the gene product negatively regulates intramembranous ossification of the mandible. This is the first case of an AFD syndrome with right ventricular cardiac malformation in which an underlying genetic cause was identified.

**P02.006****Aglossia-adactyly syndrome with mental retardation**

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Aglossia adactyly syndrome is characterized by the presence of aglossia, adactyly and various malformations of the cranium, face and limbs. Craniofacial anomalies include microstomia, micrognathia, hypoglossia, variable tongue deformations, mandibular hypodontia, cleft palate, cranial nerve palsy, broad nose, telecanthus, lower eyelid defects, and facial asymmetry. The limb defects are represented by hypoplasia/absence of the distal phalanx, total adactyly, partial limb amputation, and syndactyly. Limb defects usually involve all four limbs. Motor development are almost always normal, mental retardation is a very rare occurrence in this syndrome. A 1 month-old-male patient referred to our unit, because of congenital aglossia. His perinatal history was unremarkable. On initial physical examination, his head circumference, weight and height were all in normal range with 40 cm, 3.5 kg and 54 cm, respectively. He had prominent nose, frontal bossing, down-slanting palpebral fissures, large ears with hypoplastic antihelix, microretrognathia, aplastic tongue, and bifid uvula. Metacarpal bones were hypoplastic on his right hand. In his left hand, there was clinodactyly and tips of finger were beaked. The distal phalanx of second, third and fourth fingers, nail of second finger and toes on both feet were aplastic. On follow-up period of this patient, speech therapy was started and reconstructive plastic surgery for aglossia was planned for preschool years. His mental status appeared to be retarded, and IQ was found 53, when he was 6 years old. Here, we report a rare event of mental retardation for aglossia-adactyly syndrome.

**P02.007****When is genetic testing helpful in Alport's syndrome?**H. Hanson<sup>1</sup>, H. Story<sup>2</sup>, J. Pagan<sup>2</sup>, F. A. Flinter<sup>1</sup>;<sup>1</sup>Clinical Genetics Department, Guy's & St Thomas NHS Foundation Trust, London, United Kingdom, <sup>2</sup>DNA laboratory, Guy's & St Thomas NHS Foundation Trust, London, United Kingdom.

Alport syndrome (AS) is a predominantly X-linked hereditary nephritis associated with high-tone, sensorineural deafness and characteris-

tic eye signs. The gene frequency is 1 in 5000 and it causes 0.6% of chronic renal failure in Europe.

Most cases (85%) of AS result from mutations in the X linked collagen gene COL4A5; mutations in the autosomal genes COL4A3/A4 on chromosome 2 account for others. Mutation analysis of COL4A5 has been available in a service setting for several years; mutation detection rates using a combination of direct sequencing and MLPA now exceed 95% in patients with classical clinical signs and an X-Linked pedigree.

Questionnaires about the number of clinical diagnostic criteria that families fulfilled were sent to clinicians of 250 patients whose DNA was received for diagnostic testing; predictive tests, duplicate family members and samples that were only partially screened were excluded. One hundred and fifty two (61%) were returned. Seventy four patients (49%) had a pathogenic COL4A5 mutation.

The mutation detection rate in families fulfilling 0, 1, 2, 3 or 4 diagnostic criteria was 0%, 8.6%, 57%, 78% and 67% respectively. Twenty five (64%) of patients with COL4A5 mutations apparently meeting only 2 diagnostic criteria had incomplete clinical assessments.

In patients meeting 4 diagnostic criteria without an identified COL4A5 mutation, autosomal inheritance was confirmed or suspected in three. We recommend COL4A5 analysis in any patient meeting at least 2 diagnostic criteria. COL4A3 and COL4A4 analysis can be considered subsequently if a COL4A5 mutation is not detected.

**P02.008****Delineation of a lethal autosomal recessive disorder characterized by alveolar capillary dysplasia and limb anomalies**

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Alveolar capillary dysplasia (ACD) is amongst the common causes of lethal primary pulmonary hypertension (PPHN) in the newborn. It is ultimately unresponsive to inhaled nitric oxide and ECMO. Definitive diagnosis requires lung biopsy, and is characterized by a reduction in number of capillaries, with a decreased blood-air interface. The deficit likely involves the pseudoglandular and canalicular stages of lung development. Over 100 cases have been reported.

The etiology of most cases is unknown, but many familial cases have been reported. Associated malformations are seen in 50% of patients, recurrent anomalies involve the cardiovascular, genitourinary and gastrointestinal systems. Mutations in *STRA6* have been identified in patients with anophthalmia and malformations including ACD. The molecular basis for the majority of children with ACD is unknown. One study excluded mutations in two candidates: *BMPR2* and *EMAPII* (Sen et al, 2004).

We have encountered 4 children born to consanguineous parents (2 different ethnic groups) that presented with limb reduction anomalies and died of PPHN as newborns. In those who had autopsy, ACD was confirmed. Review of the literature identified 4 other reports of 6 children with limb reduction anomalies and ACD (Cullinane et al, 1992; Simonton et al, 1993; Steinhorn et al, 1997; Witters et al, 2001). Therefore, we are aware of 10 patients with ACD and limb reduction anomalies. Given consanguinity in 4 families, this is likely an autosomal recessive condition. This paper will include a clinical delineation of this syndrome, as well as current data on molecular studies on these families.

**P02.009****Clinical characterization of a microdeletion syndrome in Xq22.3 in a Czech family**V. Horinova<sup>1</sup>, V. Vranova<sup>2</sup>, P. Kuglik<sup>2</sup>;<sup>1</sup>Genetic ambulance and counseling, Jihlava, Czech Republic, <sup>2</sup>Department of Genetics and Molecular Biology, Faculty of Science, Masaryk University, Brno, Czech Republic.

An X-linked recessive syndrome (AMME), characterized by the Alport syndrome, mental retardation, midface hypoplasia and elliptocytosis associated with a microdeletion in Xq22.3 and explained by mutations in the COL4A5 gene was reported by Jonsson et al (J. Med. Genet. 1988, 35:273). We have identified a family with two affected male (maternal uncle/nephew) members showing clinical features similar to AMME, which was associated with an Xq22.3 microdeletion observed also in the female members of the family. The aims of this work were to compare the phenotypes of our patients with these cases and to

correlate their complex clinical manifestations with the content of the deleted chromosomal segment. Detailed examinations showed that both Czech patients were identical in terms of clinical symptomatology and they shared the major characteristic clinical features with the originally reported AMME phenotype, although the two phenotypes were not completely identical. It thus seems that a very similar contiguous syndrome associated with a microdeletion in Xq22.3 occurred independently in two unrelated families. A detailed cytogenetic comparison of the two microdeletions could contribute to clarification of the minor clinical differences observed and to the definition of specific candidate genes. Our knowledge of the phenotype of the 20 year-old uncle might also be useful for predicting the development of the symptoms in younger affected patients.

#### P02.010

##### Association of variants T2373G, T1808A glucocorticosteroids gene NR3C1 and C3435T multidrug resistance gene (MDR1) with severity of bronchial asthma (BA)

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The aim of study was to evaluate the incidence of T1808A (exon 5), T2373G (exon 9) NR3C1 gene (associated with primary glucocorticosteroidresistance - PSR) C3435T (exone 26) MDR1 gene and their association with BA severity.

88 patients with moderate and severe BA and 53 healthy controls were investigated by PCR-RLFP method.

All patients were homozygous at T allele of NR3C1 gene, so PSR was not revealed.

Rates of C allele ("aggressive allele") and T gene MDR1 in BA group were 0,52 and 0,48, in controls 0,3 and 0,7. T allele was more frequent in controls than in BA ( $\chi^2 = 12,1$ ;  $p=0,0005$ ). In patients with moderate BA T allele of MDR1 gene was more frequent than in severe BA ( $\chi^2 = 4,6$ ;  $p=0,038$ ). Rate of CC genotype of MDR1 gene was more in patients with severe BA than in controls  $\chi^2=8,15$  ( $p=0,004$ ). Incidence of TT genotype of MDR1 gene was more in patients with moderate BA than in severe one ( $\chi^2 = 4,6$ ;  $p=0,038$ ).

In 33 BA patients complications of glucocorticosteroid treatment (GCST) were observed. TT genotype of MDR1 gene was more frequent in patients without GCST complications ( $\chi^2=4,19$ ;  $p=0,0406$ ).

So, association of T2373G, T1808A variants of NR3C1 gene and MDR1 (C3435T) gene with BA severity degree was shown. In all patients signs of PSR were absent. Severe BA course with complications of GCST was revealed in patients with CC genotype of MDR1 gene. TT genotype of MDR1 gene was associated with moderate course of BA.

#### P02.011

##### A new case of Ataxia type 10 in a Mexican female

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Introduction: The spinocerebellar ataxia type 10 (SCA 10) is an autosomal dominant disorder with cerebellar dysfunction involving ataxia, dysmetria, seizures and anticipation. SCA10 is caused by repeat expansion of pentanucleotide ATTCT in the ataxin 10 gene (ATXN10, 603516.0001), candidate region on chromosome 22q13.3, these repeats form unpaired DNA structure that works as aberrant DNA replication origin and contributes to the repeat instability and cell death, but how produces a neurodegenerative effect is unknown.

The aim of this research is to present the results of the molecular studies in a female patient affected with SCA10.

Material and Methods: A female aged 20 years old was studied clinically due to SCA10, laboratorial studies were performed with 5mL of peripheral blood were used to isolate patient's DNA by GeneCatcher Kit (Invitrogen). The molecular analysis was made by PCR with previous described primers to detect normal alleles, the PCR product was

submitted to electrophoresis in 8% polyacrylamide gel. A blank, and two normal individuals were test simultaneously as quality controls.

Results: We obtained a single band that corresponds to 19 ATTCT repeated normal allele, thus our patient corresponds to an heterozygous genotype 19/unknown.

Discussion: SCA10 mutation accounts for almost 15% of autosomal dominant ataxia in Mexicans population and in Brazilians in 1.8%. This low incidence can be explained probably by the limited molecular studies in our country.

#### P02.012

##### Clinical and genetic study of a large Dutch family with autosomal dominant restless legs syndrome

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Genetic factors play an important role in the aetiology of restless legs syndrome (RLS). Familial aggregation has been documented with up to 90% of the idiopathic cases reporting a positive family history. Several loci and association with several genes have been described for Mendelian forms of RLS.

Thirty-one family members out of three generations of a Dutch family segregating autosomal dominant RLS, were examined. Each family member underwent a general medical and neurological examination. An extensive questionnaire was completed, and RLS symptoms were quantified using the International RLS Rating Scale. Serum creatinine, hemoglobin and iron levels were determined to exclude secondary RLS in this family.

RLS was ascertained in twenty-six subjects. Serum levels of creatinine, hemoglobin and iron all were in normal ranges. Mean age at examination was fifty years (20-93). The average age at symptom onset was 17.8 years (6-35), which is younger (17 versus 27) as described in literature. RLS symptoms were quantified using the RLS rating scale, with a mean initial score of 12 (0-29) and 12,3 (0-29) after two years. In this family the phenotype is very heterogeneous, including vanishing RLS symptoms in 2 patients. A genome-wide scan for linkage is in progress using the Affymetrix GeneChip® Human Mapping 250K Array set. Affected-only linkage analysis is performed considering the patients with a severe or stably moderate RLS rating scale score as "affected". Due to the large pedigree size and early-onset phenotype, this family has great potential for linkage analysis and the results will be presented.

#### P02.013

##### Heterozygous deletion of the PITX2 gene in a patient with Axenfeld Rieger Syndrome

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Axenfeld Rieger Syndrome (ARS) is a genetically heterogeneous disorder mainly characterized by developmental defects of the anterior chamber and extraocular anomalies. ARS is inherited as an autosomal dominant trait with high penetrance and has been linked to different chromosomal loci. Two genes - PITX2 and FOXC1, which both encode developmental transcription factors and map to chromosomes 4q25 and 6p25, respectively - are the most frequently associated. PITX2 mutations show great variety, from point mutations to microscopic or submicroscopic deletions, and apparently balanced translocations in few cases. Microscopic or submicroscopic deletions of PITX2 are known to result in ARS through haploinsufficiency but only in few cases the extent of the deletion was characterized by molecular means. Quantitative genomic polymerase chain reaction is the method of choice to analyze patients in which sequencing did not reveal putative mutations as the resolution limit of FISH or array-CGH is too low to detect mid-size deletions. We identified a cytogenetically invisible submicroscopic deletion at 4q25 in a patient diagnosed with ARS applying quantitative genomic PCR analysis. Further genotyping of the patient and her parents with polymorphic microsatellite markers showed that the maternal allele was not transmitted indicating a de novo deletion event in the patient. The deletion comprised the entire sequence of the PITX2 gene but did not include adjacent genes.

Recent mutation reports indicate that mid-size gene deletions might be a rather frequent cause of ARS. Therefore, analyses for mid-size gene deletions should be routinely performed in mutation screenings in ARS patients.

#### P02.014

#### Abnormal Expression of p63 (TP73L) in Bladder Exstrophy-Epispadias Complex

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The bladder-exstrophy-epispadias-complex (BEEC) is a spectrum of congenital anomalies of the lower abdominal wall, bladder, anterior bony pelvis, and external genitalia, ranging from isolated epispadias (EP), to classic bladder exstrophy (CBE), to cloacal exstrophy (CE; its most severe form). The etiology of BEEC is unknown but there is clear indication of genetic factors contributing to this birth defect. *p63*, a *p53* homolog located at chromosome 3q27-q29, was found to result in a range of developmental defects of the skin and limbs as well as bladder exstrophy in knockout *p63*-/- mice. Furthermore, *p63* gain of function mutations in humans cause genetic syndromes that frequently present with urogenital anomalies. Although obvious mutations were not detected, global dysregulation of variable *p63* isoforms is apparent in our results. Our data conclusively implicates *p63* in the etiology of BEEC. In eight out of 14 samples, we demonstrate expression differences among *p63* isoforms, including a total subclass of isoforms. Direct sequencing is in progress to rule out regulatory region mutations. We also identified a set of genes that are differentially expressed (DE) between normal and exstrophic bladders, one of which is *p63* (significantly underexpressed in BEEC bladder). *p63* downstream effector, *Perp*, is an integral part of desmosome and many other genes in the DE set (~30%) were also found to play a role in desmosomal assembly and/or cell-cell connectivity. Consistent with the suggested multifactorial inheritance of this birth defect, we propose that abnormal *p63* expression is one of the etiologic factors for this condition.

#### P02.015

#### BPES Syndrome Ohdo type in child - case report

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Background: BPES (Blepharophimosis-Ptosis-Epicantus inversus Syndrome) is an autosomal, rare and complex dominant malformation of the eyelids that may severely impair visual function. BPES locus was assigned to 3q23. BPES syndrome Ohdo type is extremely rare. It is characterized by BPES anomalies and in addition neurological anomalies, growth retardation, congenital heart disease, abnormal ears and clynodactily of fifth fingers are noticed.

Material and methods: We present a five-year and four months old male infant admitted for abnormal phenotype. The patient underwent a complete pediatric, ophthalmologic and neurologic evaluation.

Results: No similar case was known in the family. Pregnancy was uncomplicated and the child was born at 38 weeks of gestation with normal weight and length. In the first year of life seizures are mentioned. The following facial characteristic features were noticed at clinical examination: short eyelids, blepharophimosis, ptosis of the upper eyelids, epicantus inversus, telecanthus, arched eyebrows, flat, broad nasal bridge, protruding ears. Limbs defects: clynodactily of fifth fingers, flatfeet and genu valgum were present. Systolic cardiac murmur, mild mental retardation and growth delay (15 kg weight, 100 cm height and 50 cm occipito-frontal circumference) were determined. Cardiac sonography found atrial septal defect. Ocular ultrasound was normal. MLPA analysis with P036C&P070 kit didn't find major mutations.

Conclusions: Child's phenotype probably represents variable expression of the Ohdo syndrome. Typical clinical features lead to establishing the diagnosis and permit the surgical treatment of eyelids altera-

tions. The prevention of visual impairment is a very important goal in this case.

#### P02.016

#### An unusual case with camptodactyly, thenar, hypothenar muscle atrophy, spasmotic pains, excessive sweating and minor facial anomalies

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#### An unusual case with camptodactyly, thenar, hypothenar muscle atrophy, spasmotic pains, excessive sweating and minor facial anomalies

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We present a fifteen years old girl with deformities in her hands and pain in the lower extremities. She was born to a consanguineous parents at term. She had minor dysmorphological features such as hypertelorism, epicanthus, long philtrum. She had a history of inguinal hernia operation when she was 5 years old. The deformities started at age 11 years old, particularly in the hands, continued with stiffness and pain in the hands and in the lower extremities. She had stiff skin, camptodactyly, weak palmar crease pattern, abnormal dermatoglyphics, thenar and hypotenar atrophy, excessive sweating. X-Rays showed flexion contractures on her fingers but no erosion was noted. No laboratory investigation was suggestive for scleroderma. Autoantibodies for rheumatoid diseases were negative. Mutation analysis for both Crisponi syndrome and camptodactyly-arthropathy-coxa vara-pericarditis syndrome were negative.

#### P02.017

#### Congenital heart defects in CHARGE syndrome patients with CHD7 mutation

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Background: CHARGE syndrome (MIM 214800) consists of a combination of congenital malformations including Coloboma, Heart defects, Atresia of choanae, Retardation of growth and developmental delay, Genital anomalies and Ear anomalies. Diagnosis criteria have been recently refined and several other features have been described such as semi-circular canals and cranial nerve anomalies. A *CHD7* gene mutation is found in 60 % of patients with a clinical diagnosis of CHARGE syndrome. Although frequently found, cardiovascular malformations (CVM) are not specific.

Methods: In our series, 85% of CHARGE patients with *CHD7* mutation have a CVM. We report on the spectrum of CVM in 65 *CHD7* mutated patients (including 15 fetuses). All patients were precisely assessed for their cardiac phenotype by echocardiography, CT scan or pathology.

Results: Conotruncal malformations (n=16), aortic arch anomalies (n=10) and atrioventricular septal defects (n=15), were the most frequent CVM. A rare association of atrioventricular septal defect and transposition of the great arteries was described in one patient. Despite the small number of mutated cases, our data suggest that hypomorphic mutations (missense, splice sites) are less frequently associated to a heart defect than nonsense and frameshift mutations.

Conclusions: Although being a minor criteria because of lack of specificity, CVM proved to be very frequent in *CHD7* mutated CHARGE patients. The neural crest cells origin hypothesis of the CVM cannot account for all observed defects. Cardiac progenitor cells of the second heart field might be implicated in embryological mechanisms leading to CVM in CHARGE syndrome.

**P02.018****Cherubism- case report<sup>1</sup>****F. F. Cionca<sup>1</sup>, L. Cionca<sup>2</sup>, C. Vizitiu<sup>2</sup>, C. Ardeleanu<sup>1</sup>:**<sup>1</sup>"Victor Babes" National Institute for Research and Development in Pathology and Biomedical Sciences, Bucharest, Romania, <sup>2</sup>Prof. Dr. Dan Teodorescu" Clinical Hospital of Oro-Maxilo-Facial Surgery, Bucharest, Romania.

Introduction: Cherubism is a rare autosomal dominant inherited disease with variable penetrance and expressivity, characterized by a benign self-limited bone dysplasia of almost exclusively the lower and the upper jaw. Patients present bilateral, painless, generally symmetrical swelling of the jaws, caused by the replacement of normal bone with pseudocystic osteolytic lesions. The disease affects pediatric population, frequently below five years of age, with slow progression of the lesions until puberty, followed by their gradual remission in early adulthood, with rare cases of residual jaw deformity. The diagnosis implicates clinical findings, associated with radiographic and histological manifestations, and is confirmed by the molecular genetic testing of SH3BP2 gene, located on 4p16.3 and affected by mutations in 80% of the patients with cherubism.

Case presentation: A 9 years old girl presented with mild, bilateral, progressive, painless and a little asymmetrical enlargement of the mandible. There were no other clinical findings in physical examination excepting abnormal teeth implantation on the lower jaw. Radiological examination revealed bilateral multicystic lesions of the lower jaw and abnormal teeth implantation. Histological examination demonstrated the presence of numerous multinucleated acidophilic giant cells randomly distributed in a fibrovascular stroma of mononuclear spindle-shaped cells. Family history identified no other members with similar lesions.

Conclusion: Clinical findings and radiographic and histological aspects of this case plead for the diagnosis of cherubism. In the future, the patient will require molecular genetic testing for confirming the diagnosis, long -term follow-up and treatment of manifestations if necessary.

**P02.019****Reno-urinary anomalies and ciliopathies: a new approach<sup>1</sup>****c. daescu<sup>1,2</sup>, i. maris<sup>1,2</sup>, i. sabau<sup>1,2</sup>, c. duncescu<sup>2</sup>, a. chirita-emand<sup>2</sup>, i. simedrea<sup>1,2</sup>, m. puiu<sup>1</sup>, t. marcovici<sup>1,2</sup>, a. craciun<sup>1,2</sup>, o. belei<sup>1,2</sup>:**<sup>1</sup>"Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania,<sup>2</sup>"Louis Turcanu" Emergency Hospital for Children, Timisoara, Romania.

Introduction: In the last ten years there has been significant breakthrough in understanding cilia and the pathology associated with cilia malfunction. Objective: The authors present 10 cases with reno-urinary anomalies which we believe are ciliopathies. Material: 10 patients with anomalies of the renal system admitted in our clinic, Nephrology Department between 01 January 2006 - 31 Dec 2008, median age was 8.9+-7.12 years (range 1.5 to 20 years), 5 girls and 5 boys. Method: In order to establish the diagnosis we used the following imaging studies: renal and bladder ultrasonography, intravenous urography, voiding cystourethrogram, magnetic resonance imaging. Results: 6 patients were diagnosed with polycystic kidney disease, 2 of them suffered nephrectomies, 3 developed renal failure and 1 presented recurrent respiratory infections (bronchiolitis). 3 patients have neural tube defects associated with urinary anomalies and 2 of them developed renal failure. 1 case associated situs inversus, hydrocephaly and vesicoureteral reflux. Discussions: Half of these children present renal failure, one girl deceased because of renal failure complications. The quality of life of the three adolescents with neural tube defects is poor. Conclusions: It is important to recognize and if it's possible to genetically diagnose ciliopathies for a better management and for improving the quality of life of these children

**P02.020****The MSX1 allele 4 homozygous child exposed to smoking at periconception is most sensitive in developing nonsyndromic orofacial clefts.<sup>1</sup>****M. H. van den Boogaard<sup>1</sup>, D. de Costa<sup>1,2</sup>, I. P. C. Krapels<sup>3</sup>, F. Liu<sup>4</sup>, C. van Duijn<sup>4</sup>, R. J. Sinke<sup>1</sup>, D. Lindhout<sup>1</sup>, R. P. M. Steegers-Theunissen<sup>4</sup>:**<sup>1</sup>University Medical Center Utrecht, Utrecht, The Netherlands, <sup>2</sup>Erasmus MC, University Medical Center, Rotterdam, The Netherlands, <sup>3</sup>Academic Hospital Maastricht, 6202AZ Maastricht, The Netherlands, <sup>4</sup>Erasmus MC, University**Medical Center Rotterdam, Rotterdam, The Netherlands.**

Nonsyndromic orofacial clefts (OFC) are common birth defects caused by certain genes interacting with environmental factors. Mutations and association studies indicate that the homeobox gene MSX1 plays a role in human clefting. In a Dutch case-control triad study (mother, father, and child), we investigated interactions between MSX1 and the parents' periconceptional lifestyle in relation to the risk of OFC in their offspring. We studied 181 case- and 132 control mothers, 155 case- and 121 control fathers, and 176 case- and 146 control children, in which there were 107 case triads and 66 control triads. Univariable and multivariable logistic regression analyses were applied, and odds ratios (OR), 95% confidence intervals (CI) were calculated. Allele 4 of the CA marker in the MSX1 gene, consisting of nine CA repeats, was the most common allele in both the case and control triads. Significant interactions were observed between allele 4 homozygosity of the child with maternal smoking (OR 2.7, 95% CI 1.1-6.6) and with smoking by both parents (OR 4.9, 95% CI 1.4-18.0). Allele 4 homozygosity in the mother and smoking showed a risk estimate of OR 3.2 (95% CI 1.1-9.0). If allele 4 homozygous mothers did not take daily folic acid supplements in the recommended periconceptional period, this also increased the risk of OFC for their offspring (OR 2.8, 95% CI 1.1-6.7). Our findings show that, in the Dutch population, periconceptional smoking by both parents interacts with a specific allelic variant of MSX1 to significantly increase OFC risk for their offspring. The article has been published in Hum Genet. 2008 Dec;124(5):525-34.

**P02.021****A new case of early-onset Cockayne/COFS syndrome in a Spanish child with mutations in the CSB gene.<sup>1</sup>****B. Gener<sup>1</sup>, A. Garcia<sup>2</sup>, C. Dalloz<sup>3</sup>, F. Sauvanaud<sup>3</sup>, A. Sarasin<sup>4</sup>, D. Pham<sup>4</sup>, V. Lauge<sup>3</sup>:**<sup>1</sup>Clinical Genetics-Pediatric Department. Hospital de Cruces, Baracaldo, Spain,<sup>2</sup>Neuropediatrics Unit. Pediatrics Department. Hospital de Cruces, Baracaldo, Spain, <sup>3</sup>Laboratory of Medical Genetics. Faculte de Medecine, Strasbourg, France, <sup>4</sup>FRE 2939. Institut Gustave Roussy, Villejuif, France.

Cockayne syndrome (CS) is a severe neurodegenerative condition with multisystemic involvement which belongs to the family of DNA repair and transcription disorders. Cerebro-oculo-facio-skeletal syndrome (COFS) has been proved to be allelic to CS but a very limited number of COFS cases have been clarified at the molecular level so far. We report on a 20-month-old female, second child of a healthy and non consanguineous family. IUGR was evident at 30 wks of gestation. The patient was born at term. She had severe congenital microcephaly and developed failure to thrive soon after birth. She had bilateral microphthalmia and congenital cataract. Clenched fingers and lower limbs rigidity were observed. Bone X-Ray showed mild platyspondyly. Her neurological development is severely retarded. She has bilateral neurosensory deafness. Brain CT scan and MRI were normal at 4 months of age. No retinopathy is present. Extensive metabolic survey and congenital infections were ruled out. In this context, minor senile-like dysmorphic features and the presence of photosensitivity suggested the diagnosis of CS at the age of 13 months. In accordance with the clinical picture the studies on skin fibroblasts of the recovery of RNA synthesis after UV exposure showed a high deficiency and two different mutations in CSB gene (p.Leu871Pro and p.Lys1172X) were found in this patient. Very few patients have been described worldwide with these particular mutations. In our opinion this case shares similarities with CS and COFS, confirming that both entities should be considered as different parts of the same clinical spectrum.

**P02.022****Craniofacial and Oro dental Features and Phenotype/Genotype Correlations in Cockayne Syndrome<sup>1</sup>****M. Rousseaux<sup>1</sup>, M. Schmittbuhl<sup>1,2</sup>, R. Mathis<sup>1,2</sup>, M. Koob<sup>3</sup>, H. Dollfus<sup>4,5</sup>, C. Dalloz<sup>5</sup>, V. Lauge<sup>4,5</sup>, A. M. Bloch-Zupan<sup>1,2</sup>:**<sup>1</sup>Faculty of Dentistry, University of Strasbourg, Strasbourg, France, <sup>2</sup>Reference<sup>3</sup>Centre for Oro dental Manifestations of Rare Diseases, Hopitaux Universitaires de Strasbourg, Strasbourg, France, <sup>4</sup>Department of Radiology, Hopitaux Universitaires de Strasbourg, Strasbourg, France, <sup>5</sup>Department of Medical Genetics, Hopitaux Universitaires de Strasbourg, Strasbourg, France, <sup>6</sup>Laboratory of Medical Genetics, EA 3949, Faculty of Medicine, Strasbourg, France.

Cockayne Syndrome is a rare autosomal recessive neurological disease caused by defects in DNA repair via nucleotide excision repair.

The incidence in Western Europe has been recently reevaluated to 2.7 per million. The main clinical features are progressive cachectic dwarfism, mental retardation with cerebral leukodystrophy, microcephaly, progressive pigmentary retinopathy, neurosensory deafness and photosensitivity. CS is caused by mutations in ERCC8 (at 5q12) for CSA and in the ERCC6 genes (at 10q11) for CSB with no genotype/phenotype correlations so far. However other genes like XPB, XPD, XPG are causative.

Caries are considered as a minor diagnostic criteria by Nance and Berry, 1992. Other orodental features like delayed deciduous teeth eruption, malocclusion, absent/hypoplastic teeth are also described in the literature.

The aim of the present study, was to investigate the orodental findings in a series of 10 CS patients included within the PHRC 2005 "Clinical and Molecular study of Cockayne syndrome". The phenotype was documented using the D[4]/phenodent registry ([www.phenodent.org](http://www.phenodent.org)). Anomalies of teeth number (missing teeth), shape, structure with enamel hypoplasia, eruption and position (crowding) were found. The dental caries diagnostic criteria was correlated to the presence of large enamel defects prominent in severely affected patients and the subsequent difficulties in maintaining appropriate oral hygiene. Cephalometric data and analysis demonstrate a transverse hypodevelopment of the craniofacial region as well as a class II skeletal profile with vertical growth and posterior rotation of the mandible inducing an increase of the lower face height and a retrusive chin. No specific genotype/phenotype correlations were elicited.

#### P02.023

##### Cockayne Syndrom, Case Report

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Back ground: Cockayne syndrome is a very rare genetic disorder with a recessive autosomal inheritance characterized by dwarfism, microcephaly, mental retardation, deafness, a photosensitive dermatitis and a peculiar form of retinal pigmentation..

Case report: We report a case of cockayne syndrome in four year old Iranian female child. She was born of a nonconsanguineous marriage. She is mental retard and dysmorphic, Cachexia (height and weight both were below the 5<sup>th</sup> percentile).

She has failure to thrive, short stature, premature aging, microcephaly, dysarthic speech , photosensitivity, and sunken eyes, dental caries. There was no blindness or deafness, and the fundus examination was showed Tape to Retinal degeneration. The karyotype was normal .Metabolic screening was normal.The Radiologic examination (x- ray) was showed biconvex flattening of vertebrae and kyphosis.The results of DNA- repair analysis is strongly reduced DNA- synthesis after UV indicates Cockaynes syndrome in this patient.

#### P02.024

##### Fourth case of Cerebral, Ocular, Dental, Auricular, Skeletal Syndrome (CODAS), description of new features and molecular analysis

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Cerebral, Ocular, Dental, Auricular, Skeletal Syndrome (CODAS, MIM 600373) syndrome is a very rare congenital malformation syndrome. This clinical entity is highly distinctive with mental retardation, cataract, enamel anomalies, malformations of the helix, epiphyseal and vertebral malformations and characteristic dysmorphic features. To date, three affected children have been reported since 1991. The aetiology and pattern of inheritance of CODAS syndrome is still unknown. We report a new sporadic case presenting with all the characteristic features of CODAS syndrome associated with undescribed malformations including heart, laryngeal and liver malformations. All investigations such as karyotype, metabolic screening and CGH array were normal. We discuss and investigate differential diagnosis.

#### P02.025

##### MLPA analysis detects an high percentage of mutated alleles in patients affected by Cohen syndrome

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Cohen syndrome is a rare autosomal recessive disorder with variability in the clinical manifestations. It is characterized by mental retardation, postnatal microcephaly, facial dysmorphisms, ocular abnormalities, and intermittent neutropenia. Mutations in the COH1 gene have been found in patients with Cohen syndrome from different ethnic origins. In a cohort of 19 patients, we previously identified 11 mutations by the combined use of DHPLC, sequencing analysis and Real Time PCR probes covering the entire length of COH1 gene (Katzaki E, 2007 J.Hum.Genet. and unpublished data). The use of an MLPA probemix for COH1 was crucial in identifying missing mutated alleles and in defining the extension of the rearrangements. Thus, we were able to identify the second mutated allele in 2 unrelated patients and in 2 affected sisters. We also found 2 intragenic duplications spanning from exons 4 to 13 and from exons 21 to 29, respectively. This is the first time that duplications are reported in Cohen syndrome patients. MLPA was also implemental in defining the extent of 4 deletions already detected by Real Time PCR. Although in our small cohort of patients, we can consider that MLPA is able to detect 24% of mutated alleles. In conclusion, MLPA demonstrated to be an essential tool for the detection of microdeletions/microduplications within the COH1 gene at relatively low cost. For this reason we consider that it could be used as an initial screening method for the molecular analysis of patients affected by Cohen syndrome.

#### P02.026

##### Amelogenesis Imperfecta and Cone Rod Dystrophy CORD

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The recessively inherited association of CORD with amelogenesis imperfecta (AI) or Jalili syndrome (OMIM 217080) was first reported in a large consanguineous Arabic family from the Gaza strip (Jalili and Smith, J Med Genet. 1988;25:738-740). Recently the causative gene CNNM4 (2q11), encoding a putative metal transporter, has been identified in a pooled cohort of 7 unrelated families. We report and compare here the orodental phenotype, analyzed using the Diagnosing Dental Defects Database D[4]/phenodent system, in 4 individuals belonging to 2 distinct pedigrees: a two generation Kosovan family (1) and a Turkish family (2). The identified mutations were c1312dupC; Leu438ProfsX9 (1) and c.586T>C; Ser196Pro (2).

The retinal dystrophy was of early-onset, presenting in the first few years of life with photophobia and nystagmus. Visual acuity was severely reduced in childhood and there was complete absence of color vision. The dental phenotype co-segregating with the CORD was that of hypoplastic/hypomineralised AI affecting both the primary and permanent dentitions. The teeth looked dysplastic and yellow/brown with almost no visible enamel leading to severe teeth erosion/abrasion. Radiographic examination revealed absence of distinct contrast between enamel and dentine, taurodontic first permanent molars and root formation anomalies. Dentin formation seems also to be altered with the following defects (intrapulpal calcification of primary molars, bulbous appearance of permanent teeth crowns, thin roots). Strikingly

the amelogenesis imperfecta appearance is similar in all examined patients and could be considered as a real diagnostic marker for this rare disease.

#### P02.027

##### Congenital Ichthyosis - Case Study

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Introduction: Congenital Ichthyosis is a rare disorder in medical practice. It is characterized by a profound thickening of the keratin layer in fetal skin.

##### Material and Methods:

We are presenting a case of a premature newborn, female, hospitalized in our Department at the age of six days.

Results: Clinical and paraclinical examination are leading us to congenital ichthyosis. From personal history we found out the gestational age of 37 weeks, birth weight of 2430 g, Apgar score 7, born through cesarean section, oligoamnios. Mother presented urinary tract infection during the pregnancy, fever at 5 month of pregnancy.

Clinical examination emphasize: dry, fissured teguments, generalized exfoliation, bilateral ectropion, upper and lower limbs with retractions at the level of fingers, finger hypoplasia at the level of lower limbs.

There were monitored urea, serum protein level, levels of creatinine, hemoglobin, serum electrolyte, WBC count, blood cultures. Skin cultures was positive with E. Coli with favorable evolution under antibiotic therapy. Transfontanelar ultrasonography revealed a picture of intrauterine chronic affection.

Conclusions: There is a high risk of systemic infection in congenital ichthyosis; this is the main cause of death.

Under the set therapy the evolution was slowly favorable.

Congenital Ichthyosis is a rare disorder in medical practice and needs cooperation between neonatologist, dermatologist and medical geneticist.

#### P02.028

##### Clinical findings, MR imagings and cytogenetic studies in a series of 45 corpus callosum dysgenesis patients, born to consanguineous parents

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We report 45 corpus callosum dysgenesis patients (from 42 families), born to consanguineous parents. These patients were referred to our clinic because of neurodevelopmental delay and/or multiple congenital anomalies. We classified the patients according to their clinical picture (facial dysmorphism, cognitive functions, seizures..etc ), cranial MRI, and cytogenetic findings. Among 45 patients, 4 had total agenesis of corpus callosum, and the rest had hypogenesis or hypoplasia of corpus callosum. Addition to collasal dysgenesis, cranial MR imaging of the patients revealed periventricular leukomalacia, delayed myelination, gray matter pathologies (eg. pachygryria, heterotopia), Dandy Walker malformation and encephalomalacia. All patients had neurodevelopmental delay or mental retardation, and nearly half of them had seizures. Cytogenetic studies revealed 46,XY,+ (21q) karyotype in one patient with corpus callosum agenesis, characterized by epileptic seizures and he also had severe neurodevelopmental delay. As genetic etiology of corpus callosum dysgenesis is not clear, categorizing patients according to genotype and phenotype, would greatly help in research studies for etiological background. We conclude that this categorisation would probably lead to further genetical studies, such as array CGH and linkage analysis, ultimately aiding in finding out the responsible gene(s), and genetic mechanisms underlying corpus callosum dysgenesis.

#### P02.029

##### Congenital craniolacunia without any associated developmental defect

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Congenital craniolacunia is a rare calvarial ossification defect characterized by multiple, 'soap-bubble' areas of bone rarefactions, resulting in the faulty impression of abnormal calvarial openings. Lacunar skull develops during fetal life, becomes manifest at birth, and usually disappears by the 5th month of postnatal life. The mechanism underlying craniolacunia is only partially understood, and it is generally related to dysplastic bone formation associated with focal dural defects. Craniolacunia can occur in patients with spinal or cranial dysraphism and Arnold-Chiari malformation type II. Only a few cases of isolated craniolacunia have been reported.

We investigated a 6-month-old male born to healthy parents of Lithuanian origin. He was born at term of an uneventful pregnancy. Mild cranial asymmetry was noticed at birth prompting to an X-ray, which disclosed pronounced lacunar skull defects in the absence of any associated additional anomaly. Plain X-rays of the whole skeleton, neurosonoscopic, cardiosonoscopic, abdominal sonoscopic, ocular fundus evaluation were unremarkable. Psychomotor development was normal and craniolacunia was vanishing by 6 months of life.

In conclusion, isolated congenital craniolacunia is considered a rare calvarial ossification defect. However, since this defect disappears with age it is likely overlooked.

#### P02.030

##### Mixed maternal Iso- and Hetero-disomy for chromosome 7 manifesting as Silver-Russell-like phenotype with cystic fibrosis

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Maternal UPD7 accounts for 5-10% of cases of Silver-Russell syndrome (SRS) but is a rare cause of cystic fibrosis (CF). To date, only 5 cases of CF due to matUPD7 have been published. We report a sixth case that resulted from crossing over, followed by nondisjunction during meiosis II, resulting in mixed maternal iso- and heterodisomy for chromosome 7. A one-year-old male child was referred for evaluation of failure to thrive, global developmental delay and mild hypotonia. The physical examination revealed features suggestive of Silver-Russell Syndrome (SRS). Routine investigations yielded normal results, except for an elevated sweat chloride. Molecular testing demonstrated that the child was homozygous for the  $\Delta F508$  mutation. Parental testing showed that only the mother carried this mutation. Because of features suggestive of SRS, testing for UPD 7 was undertaken. Maternal UPD of the entire chromosome 7 was demonstrated: 32 of 47 microsatellite markers were informative. No paternal alleles were detected. Two regions of maternal heterodisomy were identified (7p14.3-pter and 7q22.1-q22.2) and, and by 9 markers between 7q22.1 and 7q22.2. The 13 remaining markers were suggestive of maternal isodisomy at the peri-centromeric region of the short arm (centromere to p14.1) and both the proximal portion long arm (q11.21 to q21.3) and its terminal portion (q22.3 to qter). These results are compatible with crossing over, followed by nondisjunction during meiosis II, resulting in mixed maternal iso- and heterodisomy for chromosome 7. Isodisomy at the CFTR locus resulted in homozygosity for the  $\Delta F508$  mutation.

#### P02.031

##### Convergence of molecular genetics and modern cytogenetics in clinical diagnosis

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Increasingly the scale or resolution of investigations through cytogenetics and molecular genetics are converging; driven by the elucidation of more complex rearrangements involved in human disease and the available technology. Recently the cytogenetics and molecular genetics departments made changes to clinical practice whereby patients were screened for several disorders using MLPA, prior to further studies and / or confirmation using cytogenetics methodologies (primarily Fluorescent In-Situ Hybridisation). Analysis for a range of disorders including general screening for microdeletions and duplications across all chromosomes through to specific deletions associated with phenotypes such as Smith-Magenis, Cri du Chat and DiGeorge Syndrome was performed using one or more multiplex probe ligation-dependent

amplification (MLPA) probe mixes. Thereafter, positive or inclusive results are referred to cytogenetics for confirmation through FISH. To date, 350 patients have been reported across 17 separate phenotypes using 9 separate MLPA probe mixes (supplied by MRC Holland). Of these patients 15 (4%) have been reported with a pathogenic deletion or duplication associated with DiGeorge Syndrome 22q11 (4), telomere / subtelomere (7), Williams Syndrome 7q11.23 (1), Rubinstein-Taybi Syndrome 16p13.3 (1) and Prader Willi (2). The effectiveness of this strategy when considering the concordance of results, the limitations of the methodologies, turnaround times and overall cost-benefit will be presented.

#### P02.032

##### Evaluation of three patients with disorders of sexual development

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Disorders of sexual development represent a clinically heterogeneous group of conditions that interfere with normal sex determination and differentiation. The manifestations in affected individuals may fluctuate from the neonatal period to adulthood. Disorders of sexual development arise from chromosomal, genic, gonadal, or anatomic abnormalities in the pathway of sexual differentiation, occasionally being associated with other malformations. We report clinical, hormonal and other parameters, together with the karyotype, FISH and molecular analysis in three cases born with disorders of sexual development. One case had facial dysmorphism suggesting an autosomal chromosomal abnormality. Surprisingly, cytogenetic investigation revealed a 47, XXY karyotype in all metaphases. Another case had ambivalent external genitalia and chromosomal investigation showed male genetic sex. Exploratory laparascopy and histopathological examination revealed that gonads were exclusively testes. As all the investigations led to the diagnosis of 46,XY disorder of sexual development, adequate hormonal and surgical treatment was initiated. The third case was also born with ambiguous external genitalia, chromosomal analysis together with FISH and molecular analysis for SRY region revealing female genetic sex. Clinical and hormonal data led to the diagnosis of 21-hydroxylase deficiency. Ambiguous genitalia do not occur in all disorders of sex development, but whenever ambiguity is present, it needs immediate action, to try to establish the diagnosis and to decide the best choice for the sex of rearing. A multidisciplinary approach, with a patient-centered model of care, is necessary in order to reach a prompt and correct diagnosis and treatment.

#### P02.033

##### Partial distal aphalangia, duplication of metatarsal IV, microcephaly and borderline intelligence: a third patient suggesting autosomal recessive inheritance

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Partial distal aphalangia, duplication of metatarsal IV, microcephaly and borderline intelligence (OMIM %600384) has been reported twice previously [Martinez-Frias et al.; 1995, Di Rocco et al.; 2002]. We describe a third patient with the same condition, from a family with parental consanguinity. The patient is a 10 year-old boy referred for oligodactyly. He was born at term with a birth weight of 3500 gr to healthy first cousins, as their first and only child. On physical examination, he was tall and obese, his height being 150 cm (90-97th centiles), his weight 52.5 kg (over 90th centile) and his head circumference 52 cm (3rd-10th centiles). Fingers were structurally normal. However, there were three toes on the left and four on the right with partial cutaneous syndactyly between 2nd and 3rd. Both halluces were normal structurally, but the distal phalanx of the left hallux assumed a valgus position. X-rays showed duplication of both metatarsal IVs. On the right, last toe overrided the 5th and the bifid 4th metacarpals, and had 3 proximal phalanges lying over these metacarpals. On the left, 2nd and 4th metatarsals were rudimentary, the fourth being partially duplicated. Last toe overrided the 4th and the 5th metatarsals. X-rays of hands were normal. His IQ was calculated as 50. The pedigree of the first family suggested an autosomal dominant pattern of inheritance whereas the second report suggested an autosomal recessive pattern. The present patient may support the existence of the latter pattern.

#### P02.034

##### Severe autosomal dominant dyskeratosis congenita (DC) due to *TINF2* gene mutation

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DC represents a genetically heterogeneous disease which may result from the three conventional mendelian mode of inheritance: X-linked recessive to mutation in dyskerin (*DKC1*) gene; autosomal recessive (biallelic mutations) in *NOP10*, *NHP2* or *TERT* genes and autosomal dominant (heterozygous mutations) in *TERC*, *TERT* or *TINF2* genes. *TINF2* is member of genes encoding the shelterin complex of proteins identified mutated in sporadic patients with severe DC. *TINF2* gene accounts for up to 11% of patients with DC (1). Affected patients developed dermatologic signs (leukoplakia, nail dystrophy, abnormal skin pigmentation) with bone marrow failure syndrome.

We report the natural history of a 14 year-old girl, second child from healthy consanguineous parents. She developed aplastic anemia at the age of 3 yrs and had from her sister HLA homologous bone marrow transplant. Her intelligence and development were normal. She had few premature grey hairs, small area of alopecia, oral leukoplakia, right peripheral retinal ischemia, localized keratitis, nail dystrophy with pterygia, large dyskeratosis on the perineal region and hypoplastic dental roots. She didn't have poikiloderma, cerebellar hypoplasia, brain anomaly nor any digestive problem. Graft versus host disease was suspected but dermatologic features are not found so that dyskeratosis congenita was diagnosed. Molecular investigation ruled out mutation in *DKC1* or *TERC* genes. Combined DHPLC and direct DNA sequencing of *TINF2* gene identified a c.847C>G (p.Pro283Ala) mutation in the index patient and was absent in both parents. The molecular diagnosis allowed precise genetic counseling to the family.

(1) Savage S.A. et al. *Am J Hum Genet* 2008;82:501-509

#### P02.035

##### Congenital cytomegalovirus infection as one of the main causes of early onset sensorineural hearing loss; case report

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Cytomegalovirus (CMV) infection is one of the most common congenital infection, the overall birth prevalence is estimated to ~0,64%. Only 11% of infected infants have non-specific symptoms at birth. Congenital CMV is an important but underestimated cause of hearing loss (HL). Children with congenital CMV have a risk 15-22% of developing sensorineural HL.

We report a term-born girl, birthweight 3075 g, length 53 cm. Since first year of the life she had speech delay and bilateral profound sensorineural HL. At the DNA investigation, which covers 201 mutations in 8 genes we didn't find any mutations causing HL. Brain MRI showed non-progressive leukoencephalopathy. The congenital CMV infection was diagnosed by using PCR testing to detect CMV DNA on neonatal screening card.

HL occurs up to 22% of children with congenital CMV and HL can be from mild to profound. The severity of the neurologic impairment of patients with congenital CMV is highly variable and neuroimaging studies have revealed multiple anomalies. The white matter abnormalities occur up to 22% of congenital CMV patients. In the differential diagnosis of patients with white matter lesions and sensorineural hearing loss, one should consider congenital CMV infection. Congenital CMV infection is one of the most important causes of hearing loss in young children, second only to genetic mutations. The CMV DNA analysis from neonatal screening card of Estonian children with HL of unknown aetiology is in work and results will be presented (102 cases from group of 219 patients with early onset HL).

**P02.036****Mutation analysis of Epidermolysis Bullosa in the Czech Republic**B. Jerabkova<sup>1</sup>, H. Buckova<sup>2</sup>, K. Vesely<sup>3</sup>, K. Stehlíkova<sup>1</sup>, L. Fajkusova<sup>1</sup>;<sup>1</sup>University Hospital, Centre of Molecular Biology and Gene Therapy, Brno, Czech Republic, <sup>2</sup>University Hospital, Department of Pediatric Dermatology of 1st Pediatric Clinic, Brno, Czech Republic, <sup>3</sup>St. Ann's Hospital, 1st Institute of Pathological Anatomy, Brno, Czech Republic.

Epidermolysis bullosa (EB) is a rare genetic disease characterized by extremely fragile skin and recurrent skin blistering after mild or none mechanical trauma. Traditionally, EB is classified in three major categories according to the level of dermoepidermal separation at the basement membrane (BM) zone: i) epidermolysis bullosa simplex (EBS); ii) dystrophic epidermolysis bullosa (DEB); and iii) junctional epidermolysis bullosa (JEB).

EB is diagnosed by evaluation of clinical findings, transmission electron microscopy of skin samples, and immunohistochemical mapping of protein components of dermal-epidermal junction zone. Molecular-genetic diagnostics of DEB and EBS was initiated in our laboratory in 2004.

Dystrophic EB (EBD) is caused by mutations in the collagen type VII (COL7A1) gene, which consists of 118 exons. EB simplex (EBS) is caused by mutations in the keratin 5 (KRT5) gene (9 exons) and the keratin 14 (KRT14) gene (8 exons).

DNA from EB patients and their relatives were screened for mutations in the COL7A1, KRT5 and KRT14 genes. Analysis was performed using PCR and direct sequencing. We could identify KRT5 or KRT14 dominant mutations in 13 out of 19 EBS families. As regards 27 EBD probands, we revealed disease causative mutations in 22 patients and screening of COL7A1 is in progress.

Determination of EB at the level of DNA has important implication for final confirmation of diagnosis, possibility of genetic counselling and early prenatal diagnosis.

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**P02.037****Associated Malformations in patients with Esophageal atresia**

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Esophageal atresia is a common type of congenital malformation. The etiology of esophageal atresia is unclear and its pathogenesis is controversial. Because previous reports have inconsistently noted the type and frequency of malformations associated with esophageal atresia, we conducted this study in a geographically defined population, evaluating the birth prevalence of esophageal atresia and associated malformations ascertained between 1979 and 2003 in 334,262 consecutive births. Of the 99 patients with esophageal atresia, 46 (46.5%) had associated malformations. These included patients with chromosomal abnormalities (8 cases, 8.1%); non chromosomal recognized syndromes including CHARGE syndrome, Fanconi anemia, Fryns syndrome, and Opitz G/BBB syndrome; associations including VACTERL (10 patients), and schisis; oculo-auriculo-vertebral spectrum; malformation complex including sirenomelia, and non syndromic multiple congenital anomalies (MCA) (21 cases, 21.2%). Malformations of the cardiovascular system (24.2%), urogenital system (21.2%), digestive system (21.2%), musculoskeletal system (14.1%), and central nervous system (7.1%) were the most common other congenital malformations occurring in patients with esophageal atresia and nonsyndromic MCA. We observed a striking prevalence of total malformations and specific patterns of malformations associated with esophageal atresia which emphasizes the need to evaluate all patients with esophageal atresia for possible associated malformations. The malformations associated with esophageal atresia can be often classified into a recognizable malformation syndrome or pattern (25.3%).

**P02.038****Focal dermal hypoplasia: two novel mutations in the PORCN gene**N. Revencu<sup>1,2</sup>, A. Destree<sup>3</sup>, B. Bayet<sup>4</sup>, M. Coyette<sup>4</sup>, J. L. Hennecker<sup>5</sup>, M. Vilkula<sup>2</sup>;<sup>1</sup>Center for Human Genetics, Cliniques universitaires Saint-Luc, Brussels, Belgium, <sup>2</sup>Laboratory of Human Molecular Genetics, de Duve Institute, Brussels,

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Focal dermal hypoplasia (FDH) or Goltz syndrome (OMIM 305600) is an X-linked dominant disorder characterized by patchy hypoplastic skin and digital, ocular, bony and dental anomalies. Recently, mutations in the *PORCN* gene, a putative regulator of Wnt signalling were demonstrated to cause sporadic and familial FDH cases.

We report on here the clinical presentation and the molecular studies in two unrelated girls with sporadic FDH: an 8-month-old Algerian girl (patient 1) and a 6-year-old Belgian girl (patient 2). Both had: normal head circumference at birth and developed progressive microcephaly with no developmental delay, patchy, hyper-pigmented or red atrophic skin lesion, abnormal extremities with syndactyly, +/- ectrodactyly, dysplastic nails, sparse and brittle hair with patchy alopecia, iris and chorioretinal coloboma.

Sequencing of the 14 coding exons and the exon-intron boundaries of the longest human splice variant of the *PORCN* gene (isoform D; NM\_203475.1) revealed novel heterozygous mutations in both children. Patient 1 had a *de novo* duplication of 11 nucleotides in exon 8 causing a frame-shift and a premature termination codon (c.762\_772dup-CAACTATTTG). Patient 2 had an A to G substitution which alters the ATG start codon (c.1A>G). The *PORCN* transcript does not have any ATG upstream of the start codon and the next in-frame ATG is in position 72. Thus, both mutations are expected to cause loss-of-function. This study expands the number of *PORCN* mutations in the FDH patients to 28. The clinical description contributes to the delineation of the FDH phenotype associated with *PORCN* mutations.

**P02.039****Female-to-female transmission of Frasier syndrome : Lessons for genetic counseling**K. Dahan<sup>1</sup>, N. Godefroid<sup>2</sup>, M. Jadoul<sup>2</sup>, O. Devuyst<sup>2</sup>, Y. Pirson<sup>2</sup>;<sup>1</sup>Centre for Human Genetics, Université Catholique de Louvain, Brussels, Belgium, <sup>2</sup>Department of Nephrology, University Hospital St Luc, Brussels, Belgium.

Frasier syndrome is defined by the association of focal segmental glomerulosclerosis (FSGS), male pseudohermaphroditism with streak gonads and risk to develop gonadoblastoma. There have been some recent reports that observed Frasier site mutations in genetically female (46XX) patients. These females seemed to have normal genital development. We present a female-to-female transmission of a Frasier intronic mutation affecting splicing of the primary transcript of Wilms tumour suppressor gene (*WT1*). The index patient developed an isolated proteinuria from the age of 3 years with normal renal function at age 5. No urogenital involvement and wilms's tumour were observed throughout the 2 years follow-up. Her karyotype performed at age 5 with informed consent was normal 46,XX. The mother originated from Belgium had proteinuria at age of 8 years and progressed into end-stage renal disease at age 17. Renal histology showed FSGS. She became pregnant when she was 22 years and was delivered at 35 weeks of gestation from a baby girl of 1800g. The occurrence of proteinuria in our proband combined with the medical history of her mother prompted us to perform genetic analysis which revealed the same Frasier intronic mutation in both mother and daughter.

Conclusion: these data illustrate that 46,XX patients affected by *WT1* mutations in the Frasier site have a conserved ovarian function and then, emphasizes the importance of genetic counseling in all female patients presenting a steroid-resistant FSGS. Furthermore, early genetic work-up allowed us to avoid a renal biopsy and an useless immunosuppressive therapy in our proband.

**P02.040****Progranulin gene mutations are infrequent cause of frontotemporal lobar degeneration (FTLD) in Polish population**M. Berdyski<sup>1</sup>, M. Kuzma-Kozakiewicz<sup>2</sup>, B. Gajewska<sup>3</sup>, H. Kwieciński<sup>2</sup>, C. Zekanowski<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, Medical University of

Warsaw, Warsaw, Poland.

Introduction: Frontotemporal lobar degeneration, the second most common form of presenile dementia, is clinically, pathologically and genetically heterogeneous disorder. Previous studies have shown that mutations in the gene for progranulin (*PGRN*) are major cause of FTLD with ubiquitin-positive brain inclusions (FTLD-U). *PGRN* mutation frequency range is 5-11% of FTLD cases worldwide, and 13-25 % in subpopulation of patient with familial FTLD.

The aim of the study: To detect *PGRN* mutations in Polish FTLD cohort.

Material and methods: The Polish patient sample consisted of 76 FTLD patients (age: 59.7±14, 24 females and 52 males). DNA was isolated from blood leukocytes using standard procedure. All *PGRN* exons with flanking intronic regions were sequenced.

Result and conclusion: DNA sequence analysis of *PGRN* revealed nine, previously reported polymorphism (six intronic and three exonic). No pathogenic mutation was found in the examined group. It could be concluded that *PGRN* mutations are infrequent cause of FTLD in Polish population.

#### P02.041

#### A 6 year old Fryns-like syndrome child without brachytelephalangy and nail defects.

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Fryns syndrome is an autosomal recessive multiple congenital anomaly (MCA) syndrome characterised by diaphragmatic hernia (CDH), ocular manifestations with cloudy cornea, coarse face, lung hypoplasia, distal limb/nail defects, and central nervous system, cardiac and genito-urinary malformations. FS is usually lethal in the neonatal period and only 11 survivors have been reported. The diagnosis of FS is based exclusively on clinical findings since no gene or locus has been identified so far. In contrast, different submicroscopic chromosome rearrangements were found in subjects mimicking FS, making array-CGH highly recommended. Even though a comprehensive description of the broad spectrum of FS clinical features has been outlined, no major mandatory feature has been found. Recently, the association of CDH with brachytelephalangy and/or nail hypoplasia were proposed as strongly suggestive of FS. We report on a 6-year-old male infant tested negative for array-CGH (resolution 75 Kb) in whom CDH was associated with coarse face, severe mental retardation, corneal leucoma, lung hypoplasia and cryptorchidism. Pregnancy was complicated by polyhydramnios. He also displayed ventricular dilatation, narrow small thorax with hypoplastic nipples, gastro-esophageal reflux, intestinal malrotation, flexion contractures, and minor digital anomalies including dorsiflexion of toes, fifth finger clinodactyly and unilateral four finger line. All these features are variably reported in FS. This patient presents many characteristics of FS in absence of distinct distal limb/nail defects, and strengthens the broad clinical variability in the rare FS long-term survivors.

#### P02.042

#### Genodermatology, a multidisciplinary approach: Overview of five years collaboration

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Since diagnosis of rare genetic syndromes is based on pattern recognition and requires specific expertise, as is the case with many skin diseases, we joined forces starting a multidisciplinary out-patient clinic "Genodermatology" in March 2004. Our goal was to evaluate patients with diagnostic questions in the combined field of dermatology and clinical genetics by the dermatologist and the clinical geneticist together.

Patients were referred by either a dermatologist or a clinical geneticist (or other specialists) and were seen by both specialists and a genetic counselor. In a 5 years time-period 245 patients were counselled. The

medical history of the patient and the pedigree were recorded. Clinical examination, followed by karyotyping, DNA-analysis, metabolic investigation and/or a skin biopsy (if indicated) were performed.

Four main groups could be discerned, based on diagnosis:

1. Oncogenetic syndromes (n= 31)
2. hypermobility/connective tissue disorders (n=47)
3. disorders involving the X-chromosome (n=11)
4. miscellaneous (n= 156) .

Medication was prescribed by the dermatologist if indicated or referral to the department of dermatology if special treatment was needed, was arranged. In case of a hereditary condition, genetic counselling was offered to family members. Our results indicate that combined efforts are worthwhile to offer these specific patients. Moreover, in various cases light could be shed on the pathogenetic mechanisms leading to a particular skin disorder.

#### P02.043

#### Screening of the DFNB3 locus: identification of three novel mutations of MYO15A associated with hearing loss and further suggestion for a two distinctive genes on this locus

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Recessive mutations of MYO15A are associated with nonsyndromic hearing loss (NSHL) in humans (DFNB3). MYO15A has 66 exons and encodes unconventional myosin XVA. Analysis of 77 *GJB2* mutation-negative Tunisian consanguineous families, segregating severe to profound recessive NSHL, revealed evidence of linkage to microsatellites for *DFNB3* in 4 (5.2%) families. In three families, sequencing of *MYO15A* led to the identification of three novel mutations: a nonsense (c.4998C>A (p.C1666X) and two splice sites c.9229+1G>A and c.7395+3G>C. The c.9229+1G>A substitution was assessed using the consensus score calculation to quantify the influence of the mutation on the formation of splicing loops. The calculated values for the altered sequence were 58.71% as compared to 75.83% for the wild type splice site eliciting an abnormal splicing process. Whereas the difference obtained for the c.7395+3G>C was less between the normal (86.49%) and the altered sequence (81.3%). These variations were not detected in 100 controls chromosomes. No mutation was found in the fourth family. A maximum LOD score of 7.14 was obtained with marker D17S953 in this family. This result suggests that there may be another gene in *DFNB3* locus. Sequencing of such big gene is a real challenge and a pre-screen of families segregating HL for linkage to *DFNB3* locus is almost needed before *MYO15A* mutation analysis. However, the putative genetic heterogeneity within this locus represents a pitfall of such pre-analysis. In conclusion, we discovered three novel mutations of *MYO15A* and our data suggest the possibility that there are two distinct genes at the *DFNB3* locus.

#### P02.044

#### Biochemical signs of hemochromatosis in a cohort of Canadian and Russian patients under 30 years old with different HFE genotype

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Hereditary hemochromatosis is one of the most common autosomal recessive disorders. This pathology is characterized by iron overload. It is thought that hemochromatosis caused by *HFE* mutations manifests in patients over 40-45 years old. The goal of this study was to investigate ferritin and transferring saturation (TS) levels in patients under 30 years old genotyped for C282Y and H63D mutations in *HFE* gene. We used 201 Canadian and 137 Russian patients. Information about Canadian patients was taken from the hemochromatosis database (Children's & Women's Health Centre of British Columbia). Russian patients had different therapeutic disorders. Among 137 Russians 24 had ferritin and TS tested. The following *HFE* genotypes (C282Y/H63D) were found: YY/HH; CY/HD; CY/HH; CC/HD; CC/HH; CC/DD in 17%, 15%; 37%; 13%; 17%; 1% (Canadian) and 0%, 3%; 6%; 28%;

60%; 3% (Russian). Difference in results can be explained by lower frequencies of mentioned mutations in Russian population and different principle of groups' formation. Analysis of genotype-phenotype correlations in combined cohort of patients showed significantly higher levels of ferritin and TS in young YY/HH patients comparing to patients with other genotypes ( $p<0.01$ ) (Table 1). There was no significant difference in ferritin and TS levels between females and males of the same HFE genotype. Thus biochemical sings of classical hemochromatosis appear in YY/HH patients much earlier than 40-45 years old.

Ferritin and transferrin saturation levels in a cohort of patients under 30 years old						
HFE genotype (C282Y/H63D)	YY/HH	CY/HD	CY/HH	CC/HD	CC/HH	CC/DD
Ferritin, $\mu\text{g/L}$	414.3 $\pm$ 136.3	185.2 $\pm$ 127.5	169.9 $\pm$ 78.5	231 $\pm$ 112.7	195.3 $\pm$ 67.4	146.1 $\pm$ 198.5
Measurement amount	35	32	75	36	42	5
Transferrin saturation	0.8 $\pm$ 0.1	0.5 $\pm$ 0.1	0.3 $\pm$ 0.0	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	0.4 $\pm$ 0.3
Measurement amount	35	33	75	34	43	5

#### P02.045

##### Medical Genetics and Epidemiology Study of seven districts of Bashkortostan Republic

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We have performed epidemiological study of seven districts (Burzyansky, Baimaksky, Abzelilovsky, Salavatsky, Archangelsky, Askinsky and Kugarchinsky) of Bashkortostan Republic. The total size of the investigated population was 225387 persons, including 170452 individuals belonging to southern- east, northern-east and northern-west Bashkir ethnographic groups. All population of seven districts was examined. The standard for medical genetic research was elaborated in laboratory of genetic epidemiology, Research Centre for Medical Genetics, Moscow. Segregation analysis has demonstrated good agreement between the observed and expected segregation frequencies for both AR and AD diseases. On the whole, population of Bashkortostan Republic is characterized by higher level of the prevalence rate of AD hereditary disorders in comparison with AR and X-linked diseases. Our study revealed significant differences in the prevalence rates of autosomal dominant, autosomal recessive and X-linked disorders in rural and urban populations. As regards the indigenous Bashkir population, the highest level of the prevalence rate was defined for AD hereditary disorders. The absolute values of the prevalence rates of AD and AR hereditary disorders in the indigenous Bashkir population are quite high and correspond to Udmurt population. The prevalence rates of all Mendelian disorders are varied: 4,49 $\pm$ 0,41 per 1000 persons in northern-east; 5,15 $\pm$ 0,24 in southern- east and 5,66 $\pm$ 0,63 in northern-west Bashkir ethnographic groups.

#### P02.046

##### An Iranian family with 9 cases of hereditary multiple exostoses in three consecutive generations

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A family consisting of an affected father and six children : three affected daughters, two affected sons and only one unaffected daughter .These children are the results of an unconsanguineous marriage. The paternal grandfather and his two daughters have been also affected. The second female child of this family was the proband case and coming for preconceptional genetic counseling because of her disease with multiple osseous overgrowths around her joints like knees and elbows, limitation in joint active movements, painful lesions and also cosmetic limb deformities. She wanted to know about the recurrence risk of this disease in her future offsprings. The clinical manifestations have become more serious in younger sibling. This 11 years old boy has more osseous overgrowths and serious deformities. The pedigree shows an AD inheritance pattern. The clinical diagnosis is compatible with Hereditary Multiple Exostoses.

The result of the molecular study on the 11 years old son of this family has shown a heterozygous c.1235G>A mutation in exon 4 of the EXT1 gene .This mutation alters a tryptophane in a premature stop codon on

aminoacid position 412 (p. Trp412X). The mutation results in a truncated EXT1 protein or diminished EXT1 mRNA due to mRNA decay. The c.1235G>A mutation is a novel mutation not previously described in other patients nor in controls. However, due to its truncating nature, it is most likely disease-causing.

#### P02.047

##### Holoprosencephaly - lobar form - associated with occipital meningoencephalocele

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Introduction: Holoprosencephaly is a type of cephalic disorder. This is a disorder characterized by the failure of the prosencephalon to develop.

##### Material and Method:

We are going to present a new born, male, hospitalized in the Prematute and Neonatology Department.

Results: The newborn is the sixth birth; 36 weeks gestational age with birth weight of 2470 g, Apgar score 6 and cranial circumference 39 cm. Personal history of mother reveals urinary tract infection and dental abscess in the first trimester of pregnancy, therefore she had antibiotic treatment.

Transfontanelar ultrasound shows complex cerebral malformation with holoprosencephaly in lobar form, agenesis of corpus callosum and lateral ventricle dilatation, confirmed also by MRI.

The newborn was operated at neurosurgery, initial for hydrocephaly and it was set a ventriculoperitoneal drain followed by the treatment of occipital meningoencephalocele.

Conclusions: Lobar holoprosencephaly, a rare disorder, has no specific clinic expressiveness; the diagnosis can be missed in the neonatal period. Cerebral imagistic was the main method for setting a diagnosis; cerebral ultrasound has detected the lesions and MRI established their dimensions.

#### P02.048

##### ZIC2 mutations in holoprosencephaly: clinical and molecular data of a European series of 40 probants (25 fetuses and 15 children)

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ZIC2 transcription factor gene is one of the four main genes implicated in holoprosencephaly (HPE), a complex brain malformation resulting from incomplete midline division of the prosencephalon. Since 1996, a European network was organized from Rennes, France, data and DNA of 500 HPE patients were analyzed and DNA prospectively collected. Here we report the clinical and molecular data of 25 fetuses and 15 children with ZIC2 mutations. HPE is mainly isolated and syndromic in only 5 cases. All three classical types of HPE are described: alobar, semi-lobar and lobar, but syntelencephaly (middle interhemispheric variant) is also reported twice, anencephaly/cleft face and a minor form once each. Interestingly, neural tube defects are associated with HPE in three cases like rachischisis and myelomeningocele. Even in severe forms of HPE, normal faces are described in 4 cases, mild facial anomalies in most of the cases and proboscis in only one case.

Molecular studies identified 36 mutations, including 31 different types of mutations, and 4 deletions of the whole ZIC2 gene. Most mutations are detected in only a single family, except for the 8 poly-Alanine tract expansion cases, and result from de novo mutations.

In conclusion, most of time, normal face or mild facial anomalies, syntelencephaly and neural tube defect associated with HPE are correlated with ZIC2 alterations.

**P02.049****TBX5 analysis in 79 Holt-Oram syndrome families reveals 80% of genomic alterations in typical cases, 25 "new" mutations and unusual modes of inheritance**

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Holt-Oram Syndrome (HOS) is characterized by the association of upper limb radial-ray and heart malformations. The transmission is autosomal dominant and *TBX5* mutations are observed in about 75% of the typical patients, so that genetic heterogeneity is likely. *SALL4* mutations are responsible for some atypical cases.

79 families (126 patients) were referred for *TBX5* analysis. Using precise diagnostic criteria we classified them in two separate groups: 46 (91 patients) were considered "typical" and 33 (35 patients) were considered "atypical".

Sequencing of exonic and intronic flanking regions of *TBX5*, as well as QMPSF and MLPA were performed. Thirty-seven anomalies (80%) were identified in the typical families (13 nonsense, 10 frameshift, 7 point, and 3 splice mutations; 3 exonic deletions, 1 exonic duplication). To our knowledge 25 of these 37 anomalies had never been reported previously. Variable clinical expression is the rule in HOS and was confirmed in our patients. Important intrafamilial variability was also noticed in one family (slight abnormal clavicle curvature in a mother and very severe radial ray defects in her two daughters). Somatic mosaicism was identified in one asymptomatic case.

In the 33 "atypical families", a precise diagnosis could be performed in 9/33 (27%). A *TBX3-TBX5* deletion in a patient presenting features of both HOS and Ulnar-mammary syndrome was identified. In the 32 remaining families, a diagnosis was achieved in 8 cases (1 Fanconi, 1 Okihiro, 1 TAR, 2 fetal valproate syndromes, and 3 array-CGH anomalies).

This large series highlights genetic heterogeneity and clinical variability in HOS.

**P02.050****Clinicopathological pattern and genetic mapping of fetal cerebral proliferative vasculopathy: the Fowler syndrome**

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Cerebral proliferative glomeruloid vasculopathy (CPGV) is a severe disorder of central nervous system (CNS) angiogenesis, resulting in abnormally thickened and aberrant perforating vessels, forming glomeruloids with inclusion bearing endothelial cells. This peculiar vascular malformation was delineated by Fowler in 1972 in relation with a familial, lethal, fetal phenotype associating hydranencephaly-hydrocephaly with limbs deformities, called proliferative vasculopathy and hydranencephaly-hydrocephaly (PVHH). Our clinicopathological study

in a series of 14 fetuses from 8 unrelated families, permits identification of a diffuse form of PGV, affecting totally the CNS that we opposed to focal forms, confined to restricted territories of CNS.

In PVHH, the disruptive impact of vascular malformation on the developing CNS is now well admitted. However, the mechanism of abnormal angiogenesis involving exclusively brain vasculature remains unknown. Recurrences and consanguinity have been reported, suggesting autosomal recessive inheritance. Among the 8 families we report, 3 are consanguineous with 2 affected sibs in each. A genome wide scan with Affymetrix SNP 250K is currently being performed in these 3 families, and will hopefully point to the Fowler syndrome locus.

**P02.051****A new phenotype in a family with inability of tongue movement, dysmorphic face, hypernasal speech, hypogonadism and syndactyly.**

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Dysmorphic face with microbrachycephaly, triangular and elongated face, fine hair, frontal upsweep, narrow forehead, bitemporal narrowing, thick and medially-flared eyebrows, prominent tubular nose, prominent columella, enophthalmos, thin lips, pointed chin findings may be related any type of a dysmorphic syndromes. Inability of tongue movement, hyper nasal speech, hypogonadism and syndactyly in addition to dysmorphic face findings in two affected cases in a consanguineous family may represent a new type of rare phenotype with autosomal recessive inheritance pattern. Here we presented a male patient which is characteristic with the limitation of tongue movement, and dysmorphic face, hypogonadism and syndactyly findings. He had 46,XY karyotype. His sister had similar clinical findings including dysmorphic face and limitation of tongue movement except syndactyly. We have not performed her hormonal analyses for the observation of hypogonadism yet. Interestingly, no EMG abnormality was detected in tongue and whole body muscles. There was no cardiac and brain defect according to ECHO cardiography and cranial tomography results, respectively. So limitation of tongue movement and other abnormalities in a consanguineous family seems as a new phenotype with autosomal recessive inheritance pattern.

**P02.052****Isolated Hereditary Hypotrichosis Research in Russia**

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Laboratory of Genetic Epidemiology RAMS in collaboration with course of the skin and venereal disorders in childhood of Russian Medical Academy of Postgraduate education study hair growth abnormalities and scalp hair loss. In collaboration with Rogaev E.I., in 2006 year we mapped phospholipase gene (*LIPH*) from identified by us 59 Mari and Chuvash families, which had isolated hereditary hypotrichosis, and described mutation - deletion of exon 4 of the *LIPH* gene. We studied frequency of this mutation in healthy individuals of different ethnosc from the Volga-Ural region: Maris, Chuvash, Bashkirs, Udmurts and Russian. At this moment, we found new mutation in *P2PY5* receptor gene in one patient who had the similar to isolated hereditary hypotrichosis, caused by deletion of exon 4 of the *LIPH* gene, phenotype and hair structure. Further, we examined one family with clinical presentation similar to hereditary hypotrichosis, but in this family we observed spontaneously symptoms regress in adult age (although symptoms did not disappear completely). Hairs show typical beaded or monilethrix appearance and elliptical nodes separated by narrow internodes without medullary layer. We examined the patient with monilethrix by means of molecular genetic tests. We detect previously known mutation in 7 exon of the *hHb6* gene. So, clinical presentation of hair growth abnormalities is similar in different gene defects. As a result, now we are working out the differential-diagnostic criteria for genetically different forms of isolated hereditary hypotrichosis.

## P02.053

## Genotype-Phenotype Correlations In Best Vitelliform Macular Dystrophy

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The Best disease has an autosomal dominant pattern of inheritance with very highly variable expressivity. Our purpose was to determine the genotype-phenotype correlation in Best VMD patients with heterozygous or homozygous mutations in the VMD2/BEST1 gene. Best VMD patients and relatives that presented consecutively at the Créteil University Eye Clinic and at the Foggia University Eye Clinic were included in this prospective study. They were evaluated prospectively regarding age, age at onset, best-corrected visual acuity (BCVA), fundus autofluorescence (FAF), fluorescein angiography (FA), optical coherence tomography (OCT), electro-oculography (EOG).

All 11 exons of VMD2/BEST1 were amplified by PCR and mutation analysis was carried out by sequencing these PCR products. All exons were screened in all probands. We identified 9 different VMD2 mutations (2 novel), in 7 unrelated families and 3 isolated cases (9 heterozygous, and 1 homozygous). Patients presented with various stages of the disease, from absence of clinically detectable lesions / previteliform lesions to end-stage lesions. The patients age ranged between 3 and 75 years. Age at onset varied between 2 and 67 years. BCVA ranged between 20/20 and 20/200. Even with the same mutation, the age at onset and the disease progression was highly variable interfamilially and intrafamilially. No association existed between the specific nature of VMD2/BEST1 mutations and expressivity, as regards age, age at onset, BCVA, and stage of the disease as evaluated by FAF, FA, OCT ( $p>.05$ ). The type of VMD2/BEST1 mutations were not associated with the severity of the phenotype in the Best VMD patients determined.

## P02.054

## Identification of the common NEMO rearrangement in Korean patients with incontinentia pigmenti

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Incontinentia pigmenti (IP) is a rare X-linked dominant disorder characterized by highly variable abnormalities of the skin, hair, nails, teeth, eyes, and central nervous system. Mutation of NEMO/IKBKG gene in Xq28 is believed to play a role in pathogenesis and it occurs mostly in female patients due to its fatality in male in utero. Although many patients have been reported worldwide, there are no genetically confirmed patients in Korea. In the present study, we performed a genetic analysis on the NEMO gene in four Korean female patients clinically diagnosed with IP. All the patients had typical clinical manifestations of IP including abnormal skin pigmentation, nail and dysplasia. Deletion of exons 4 to 10 in NEMO gene, the most common mutation in IP patients, was detected in all the patients by the long-range polymerase chain reaction (PCR) test. This method enabled us to discriminate between NEMO gene and pseudogene ( $\Delta$ NEMO) rearrangement. Therefore, a PCR-based analysis of the NEMO gene is useful to establish the definitive diagnosis of IP for a timely treatment to improve patient prognosis. Furthermore, all the patients showed completely skewed patterns of X-inactivation, indicating cells carrying the mutant X as the pathogenicity of the disease. To the best of our knowledge, this is the first report of genetically confirmed cases of IP in Korea. More investigations are needed to identify genotype-phenotype correlations and ethnicity-specific genetic background of IP.

## P02.055

## A very early juvenile Huntington disease revealed by cerebellar ataxia in a 2 years old boy

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<sup>1</sup>Inserm and Rouen University Hospital, Rouen, France, <sup>2</sup>Rouen University Hospital, Rouen, France, <sup>3</sup>Strasbourg University Hospital, Strasbourg, France. Juvenile Huntington disease (JHD) is a rare clinical entity characterized by an age of onset of Huntington disease (HD) younger than 20. JHD accounts for 1% to 10% of the HD cases. In 80% to 90% of cases, transmission of JHD is paternal. Patients with JHD have usually more than 60 CAG repeats within the *IT15* gene. We report on the case of the 4<sup>th</sup> child of unrelated parents. He was seen at the age of 23 months for absence of unaided walk and delayed speech. His development was considered as normal until 15 months of age, when an insufficient weight gain was noted and motor delay appeared. Clinical examination showed truncal hypotonia, postural and intentional tremor, lower limbs rigidity and ataxia. Cerebral MRI showed cerebellar atrophy. Extensive chromosomal and metabolic screening was normal. Six months later, his father, 47 years old, was seen for a 4 years history of progressive dementia with severe behavioural disturbance and chorea. MRI was normal. DNA analysis revealed a 43 CAG expansion of the *IT15* gene in the father and a very large expansion (more than 100 CAG) in the child. This unusual case shows that very early onset JHD due to large CAG expansions should be considered in case of motor skills delay associated to cerebellar atrophy. In addition, this observation highlights the diagnosis pitfall of JHD in young children especially if the diagnosis is not yet established in the parents.

## P02.056

## Congenital heart defects in Kabuki syndrome

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Kabuki syndrome (KS) is a congenital mental retardation syndrome with additional features, including distinctive facial features, dermatoglyphic abnormalities and short stature. Some cases associate heart defects. Most cases are sporadic. The underlying genetic mechanism remains unknown.

We have analysed the prevalence and types of congenital cardiac defects in 20 children with Kabuki syndrome recorded in the files of Iasi Medical Genetics Center. There were 7 girls and 13 boys, diagnosed at a median age of 4.9 years (ranges from 0.2 to 13 years). The diagnosis was based on the presence of the characteristic features of the disorder. Cardiac defects were present in 9 children (45%) - atrial septal defect (35%), coarctation of the aorta, aortic stenosis, bicuspid aortic valve (5%) and dextroposition (5%). The prevalence of heart defects was higher in boys (53.8%) than in girls (28.5%). Complex heart defects were present only in males. In the literature aortic coarctation, atrial septal defect and ventricular septal defect are the most frequent congenital heart defects associated with Kabuki syndrome. The comparison of our data and the literature data will be presented in detail. In conclusion we present a study of 20 cases with Kabuki make-up syndrome, 45% of them having heart defects. Cardiac defects are commonly associated to KS and males are more frequently and severely affected than females. Patient's prognosis depends on the presence/ absence of cardiac defects, renal failure and severity of mental retardation.

## P02.057

## Kabuki make-up syndrome in two Tunisian girls with consanguinity and background of mental disability

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Kabuki makeup syndrome (KS) is characterized by distinct facial anomalies, mental retardation, congenital heart defect (CHD), and skeletal malformations.

In the present study, we report two Tunisian girls, who fulfilled clinical criteria of KS syndrome, diagnosed in the course of our genetic testing strategy of CHD at the medical university of Sfax.

Patient 1 is a 9-year-old girl who had partial atrioventricular canal defect and patient 2 is a 17-year-old girl who had double outlet right

ventricle. Patient 1 had postnatal growth retardation (weight 2200 g at birth), general developmental delay and recurrent bronchitis. She had mild mental retardation with learning and social difficulties. She had also, a peculiar face characterized by eversion of the lower lateral eyelid, arched eyebrows, mild ptosis, strabismus, depressed nasal tip with prominent nose and short nasal septum, malformed ears and abnormal teeth (delayed teething at 7 years old). The patient had abnormally short fifth digits in hands, foetal fingertip pads and deformities of the feet fingers. She had also bilateral perception deafness. Patient 2 had postnatal growth retardation and hypotonia, short stature and learning difficulties. She had characteristic facial features of KS syndrome with hair abnormalities (brittle hair) and severe skeletal malformation particularly butterfly vertebrae with scoliosis.

Chromosomal studies showed a normal female 46,XX pattern with no evidence of del22q11 by FISH.

There was history of consanguinity for our 2 patients with background of mental disability associated to congenital blindness or to seizures, multiple new born deaths, celiac disease, scoliosis and male infertility.

#### P02.058

##### **Knobloch syndrome : a cause of encephalocele and vitreo-retinal degeneration.**

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Knobloch syndrome is a rare autosomal recessive disorder, described in 1971 by Knobloch and Layer, characterized by vitreo-retinal degeneration, high myopia, recurrent retinal detachment, and occipital encephalocele. In 1996, the gene was mapped to 21q22.3, in a large consanguineous Brazilian family (Sertie et al, 1996) and a mutation in the COL8A1 gene was identified in this family, and in additional families (Sertie et al, 2000).

We report here on two sibs with Knobloch syndrome.

Yacine is the second child of consanguineous Algerian parents. At 4 months of age, myopia was detected. At eleven, he had bilateral retinal detachment with vitreal degeneration and chorio-retinal atrophy. Clinical examination and psychomotor development were normal.

Salim, the fourth child, was born after an uneventful pregnancy with normal birth weight. A midline occipital meningocele was noted and confirmed by cranial resonance magnetic imaging excluding other cranial malformation. At 4 months, ophthalmological examination revealed high myopia and at 3 years he had amblyopia of the right eye and vitreo-retinal degeneration. He had delayed language skills but normal neuropsychological evaluation.

The two sibs were homozygous for COL18A1 gene region microsatellite analysis. Screening of the exons 3, 21, 23, 35, 38 and 42 of the COL18A1 gene has shown an homozygous duplication in the exon 23 : c.2118dupC.

These cases are illustrating the clinical variability of the phenotype in Knobloch syndrome.

#### P02.059

##### **Report of Knobloch-Layer (1971)-detached retina; encephalocele (autosomal recessive) from Iranian families**

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Knobloch syndrome (KS) is an autosomal recessive disorder defined by the occurrence of high myopia, vitreoretinal degeneration with retinal detachment, macular abnormalities and occipital encephalocele. Knobloch syndrome caused by mutations in the COL18A1. Clinical variability is present in the manifestation of this syndrome, but ocular abnormalities are severe, progressive and irreversible, leading to bilateral blindness. Scalp defect or occipital encephalocele is also a major clinical feature. Other minor clinical abnormalities are: lens subluxation, cardiovascular anomalies, joint hyperlaxity, genitourinary and

gastrointestinal abnormalities. We report a brother and sister with this condition. They are resulted from consanguineous marriage (first cousin once-removed), and too suffered from bilateral cataract, retinal detachment, high myopia, hypertelorism, bilateral epicanthic folds and bilateral sever choriorretinal pigmentary degeneration. They have also epilepsy, and retardation of speech and developmental milestone. Occipital true encephalocele was detected in two cases.

#### P02.060

##### **Laryngomalacia (OMIM 150280) and congenital stridor in infants**

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At present hundreds children with noisy breathing per year are investigated in one large hospital. The otolaryngologists proved that laryngomalacia provides about 70 % of these cases. About 10 % of cases are severe and required surgical intervention. The diagnostics is based on revealing of peculiar endoscopic sign: inspiratory collapse of laryngeal vestibule.

It would be naive to regard all these cases as one monogenic syndrome. This investigation includes genetic counseling in cases of endoscopically proved laryngomalacia (100 families). The precise history of stridor and all other peculiarities of patients were assessed. The genealogical method was used. The proband's parents underwent indirect laryngoscopy.

The residual deformation of larynx looked like result of laryngomalacia was found in 12 proband's mothers, 7 proband's fathers and both parents were peculiar in 3 cases (22 % at all). The anamnestic data (transitory noisy breathing) proved these findings in just 4 cases and were never present in adults with normal larynx.

Anamnestic and objective peculiarities of patients allowed forming two groups with prevalence of neurological problems (27 %) or signs of connective tissue dysplasia (73 %). Twenty (91 %) of "family" cases were revealed in second one. The worsening of stridor occurred in sleep usually in "neurological" group and under exertion in another. Conclusion: the laryngomalacia is heterogenous disorder. The genetic counseling in cases of congenital stridor is indicated and perspective.

#### P02.061

##### **Mitochondrial C11777A mutation in the fourth subunit of NADH dehydrogenase encoding gene associated with Leigh-syndrome**

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Leigh-syndrome as a subacute necrotizing encephalopathy has extensive genetic heterogeneity including mitochondrial DNA alterations that have been described previously in the background of the disease.

A 17-month-old male proband reported here was born with appropriate parameters at term. At the age of 5 days myoclonus were observed. West syndrome was diagnosed and treated with antiepileptic drug at the age of 11 months. Soon after, T<sub>2</sub>-weighted MRI of the patients depicted increased signal intensity within the mesencephalon and the medulla oblongata; moderate frontal atrophy and ventricular dilatations were also discovered. Besides, elevated lactate levels were detected in the cerebrospinal fluid. Suddenly, he died from cardiorespiratory arrest at the age of 17 months.

Postmortem analyses of the respiratory chain enzyme in the skeletal muscle revealed a defect in the complex I activity, associated with C→A transversion in heteroplasmic form at the 11777 position of the mitochondrial DNA causing Arg→Ser amino acid change that affects the fourth subunit of the NADH dehydrogenase enzyme encoding gene (MTND4).

This case represents the first report of a mitochondrial mutation associated with Leigh-syndrome in Hungarians, to our knowledge, this mutation was described in two Japanese and in one Italian patients with phenotypic variations in correlation with complex I deficiency and Leigh-disease. In our case, earlier onset of the symptoms and the aggressive outcome of the disease are highly indicative of the pathogenicity and the clinical importance of C11777A mitochondrial mutation in the development of Leigh-syndrome.

**P02.062****Leucoencephalopathy with megalencephaly, spongy degeneration and 17p deletion: A new disease?**

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This second child from non consanguineous parents was born at term by cesarean section for unexplained hydramnios. Birth parameters were at -1 SD. After minor neonatal feeding difficulties, she was investigated at 3 years of age for moderate and non progressive psychomotor retardation (with mainly speech delay) and dysmorphic features. Clinical findings included macrocephaly (head circumference +3 SD), contrasting with growth delay (weight -1 SD, height -2 SD), wide forehead, small nose and coarse facial features. There was no hepatosplenomegaly, no joints contractures and no signs of peripheral neuropathy. Basic biological findings were normal. Brain MRI disclosed bilateral and symmetric leucoencephalopathy with triventricular dilation, micro cysts in periventricular white matter and corpus callosum and Chiari type 1 malformation. Activities of hexosaminidases A and B measured in leukocytes were normal, excluding GM2 gangliosidosis. CGH-array (Agilent 44K) revealed a large (2 Mb) subtelomeric deletion of the 17p region between nt 48539 and nt 2104702, thus from RPH3AL (rabphilin 3A-like, without C2 domains) to SMG6 (non-sense mediated mRNA decay factor). The deletion encompassed 38 genes, none of which known to be involved in leucoencephalopathy. The aspartoacylase gene (Canavan) is located centromeric to the deletion. Although the deletion could be coincidental with the cerebral phenotype, our patient show («07») 's presentation suggests that the 17pter region may contain a second locus for leucoencephalopathy with spongy degeneration. Inheritance could be dominant or recessive (with haploinsufficiency by deletion for one allele, and loss-of-function point mutation for the second allele).

**P02.063****Investigations of LHON in Iranian Patients**

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Leber's Hereditary Optic Neuropathy is a rare condition which can cause loss of central vision. It usually affects men. The time when someone is losing their eyesight is often called the 'acute' period. After a few more weeks, the eyesight stops deteriorating. Although that course describes the most common pattern for Leber's, onset can be sudden or take place over a period of years. Not everyone in a family affected by Leber's will lose their eyesight, and we do not yet know how to tell who will get symptoms. Men cannot pass on Leber's Hereditary Optic Neuropathy to their children. Leber's Hereditary Optic Neuropathy is linked to a number of genes, all within mitochondrial DNA (mtDNA). We believe that the particular gene changes linked to Leber's Hereditary Optic Neuropathy lower the amount of energy available to the cells of the optic nerve and retina. These cells are damaged and may even die because of this lack of energy. 50 were analysed for mtDNA mutations by PCR, sequencing and RFLP methods for mitochondrial genome. 15 cases had a G11788A mutation, 10 cases had mutation G3460A and 2 patients had mutation T14484C, and no mutations were detected in the remainder.

**P02.064****Leber's hereditary optic neuropathy and Multiple Sclerosis: Harding's syndrome**

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The optic neuropathy Leber's hereditary, usually affects men between 20 and 30 years, although the disease can begin at any age, more rarely in women. The syndrome of Leber's due to mitochondrial DNA alterations (maternal mitochondrial inheritance). However, it is not possible to predict who may be affected even within the same family considered "healthy carrier". Was found that in Caucasian populations mutations of nucleotides 3460, 11778 and 14484 of the mitochondrial DNA are present in 95% of cases of Leber's syndrome. We describe a 33 year old man came to our medical attention complaining of weak-

ness of the lower limbs and pyramidal signs. Oligoclonal bands were present in the cerebrospinal fluid, and magnetic resonance imaging (MRI) revealed white matter lesions characteristic of multiple sclerosis (MS). DNA sample was isolated from peripheral venous blood. The patient tested for the three pathogenic mtDNA LHON point mutations: G11778A, G3460A, T14484C and G15257A by means of PCR and successive analysis of restriction with specific enzymes. Mutations were confirmed by direct sequencing. The patient was homoplasmic for the T14484C LHON mutation and heteroplasmic for the G15257A. The coexistence of multiple sclerosis and Leber's hereditary optic neuropathy (Harding's syndrome) is known to occur more often than would be expected by chance; therefore, screening for Leber's mutations in multiple sclerosis patients should be considered because this has important prognostic and genetic implications.

**P02.065****Malignant vascular phenotypes in Loeys-Dietz Syndromes associated with mutations in the TGFBR1 and TGFBR2 genes**

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Mutations of Transforming Growth Factor beta Receptor 1 (TGFBR1) and Receptor 2 (TGFBR2) genes cause Loeys-Dietz syndromes (LDS). Based on the presence or absence of cranio-facial traits the syndromes are classified as LDS1 and LDS2, respectively.

The probands of 33 families were referred to our attention for suspected Marfan Syndrome (n=26), EDS-IV (n=1) and Thoracic Aortic Aneurysm and Dissection (TAAD) (n=6) and found to carry TGFBR1 and TGFBR2 mutations. Probands and relatives underwent genetic counselling, multidisciplinary clinical and imaging evaluations and molecular analysis. Of the 33 probands, 24 were diagnosed with LDS1 (18 de novo) and 9 with LDS2 (1 de novo). Of 62 mutated family members, 41 carried TGFBR2 and 21 TGFBR1 mutations; 34 were in LDS1 and 28 in LDS2 families. LDSs shared aortic aneurysm (88% and 86%), dissection (23% and 36%), arterial tortuosity (100%) and, at a minor rate, aneurysms of other arteries (48% and 26%). The mean age at first diagnosis was 17±17 vs. 38±16 (p<0.0001) and at first aortic surgery was 23±15 vs. 43±15 (p<0.0012) in LDS1 and LDS2, respectively. During 59±68 months of follow-up, there were 40 dissection-related surgeries in 19 patients and 11 elective surgeries in 10 patients. Eight patients, first diagnosed at the time of dissection, died at surgery and one died of cerebral haemorrhage one year after elective surgery. None of the non-dissected patients died.

LDS1 and LDS2 are malignant diseases affecting cardiovascular system with high rate of aortic dissection in unrecognised patients. Only timely diagnosis prevents catastrophic events.

**P02.066****Marfan syndrome with extreme cardiovascular complications in a patient with mutations in both FBN1 and TGFBR2**

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**Introduction:** Marfan syndrome (MFS) is caused by mutations in the fibrillin-1(FBN1) gene. Recently, mutations in the TGFBR2 gene were found to cause Loeys-Dietz syndrome (LDS), MFS and familiar aortic dissections. Mutations in TGFBR2 are often associated with aggressive vascular pathology including arterial tortuosity and early dissections.

**Case report:** We describe a 39-year old male who was diagnosed with MFS at age 13. At that time, he had a dilated aortic root and characteristic skeletal and facial features. The parents and sister were healthy. At age 20, he underwent aortic root replacement, followed by surgery of the complete aorta in the years thereafter. In addition, aneurysms and dissections of multiple other arteries were found. DNA-analysis of the FBN1 gene identified a pathogenic mutation (c.5952T>A). Because of the severe vascular pathology, mutation analysis of TGFBR2 was performed as well revealing a known and probably pathogenic missense mutation (c.1159G>A).

**Discussion:** This is the first reported patient that carries pathogenic

mutations in *FBN1* and *TGFBR2*. The patient fulfils the clinical criteria for MFS, but has extremely severe cardiovascular complications, worse than observed in patients with *TGFBR2* mutations only. Thus, the molecular findings match the clinical features. *In vitro* studies investigating these two mutations and their interactions are pending, possibly providing a rationale for the choice of future medical treatment in this patient.

**Conclusion:** In selected patients with features of MFS as well as features suggestive of *TGFBR2* mutations, DNA-analysis should include *FBN1* and *TGFBR2*.

#### P02.067

##### Neuhauser syndrome with novel findings: A case report of a rare disorder

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Neuhauser Syndrome (Megalocornea-Mental Retardation) is a rare disorder associated with major findings of hypotonia, mental retardation, poor coordination, and megalocornea.

A 5 1/2 year -old-male patient referred to our department for atypical facies and autism disorder. He had operations for congenital glaucoma and cryptorchidism at 2 and 4 years old, respectively. He was severely mentally retarded. He had yellowish white hair, frontal bossing, downslanting palpebral fissures, strabismus, megalocornea, epicanthal folds, anteverted nares, thin upper lip, narrow palate, low-set ears with hypoplastic helixes. He also had long fingers, pectus carinatus, widely spaced nipples, scoliosis, kyphosis, sacral dimple, pes planovalgus. Reduced deep tendon reflexes, ataxic walking and hypotonia were evident on neurological examination. Cranial MRI revealed arachnoid cyst of posterior fossa, subependimal nodular heterotopias, and left venous angioma. Abdominal ultrasonography, blood amino-acides, urine organic acids, hearing examination were normal. Transillumination defect of iris, retinal hypopigmentation, megalocornea (right: 13 mm, left: 11 mm) and operated congenital glaucoma were apparent on eye examination. Cytogenetic analysis revealed a normal male karyotype with 46,XY. The examination findings clinically confirmed the diagnosis of Neuhauser syndrome.

In conclusion, we report a case of Neuhauser syndrome with novel findings of ventricular nodular heterotopia, congenital glaucoma, retinal hypopigmentation, and albinismus-like appearance, which developed during the course of the disease.

#### P02.068

##### Genomic aberrations in relation to unexplained mental retardation in Estonian patients

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Mental retardation (MR) affects 1-3% of population. This heterogeneous disorder is caused by genetic, epigenetic and environmental factors solely or in their combination. Despite extensive investigations, the underlying reason(s) remain unknown in approximately half of the MR patients and molecular basis of the pathogenesis is still poorly understood.

In order to find out causative factors in Estonian patients with idiopathic MR and to help to shed light on underlying molecular mechanisms, we have collected a cohort of 250 unexplained MR patients and unaffected members from 90 families.

To screen genomic aberrations potentially related to the clinical phenotype we have applied Infinium Human370CNV SNP-arrays.

In 14 out of 76 (18%) families analysed to date we have found aberrations with putative clinical significance, ranging in size from 0.5 to 8.3 Mb. These either affirm recently described microdeletion (like 15q13, 17q21.3) or microduplication (for example 7q11.23) syndromes or are less characterized regions with potential clinical significance.

More detailed characterization of these genomic regions as well as investigation how these imbalances influence expression in relations to etiology of MR is currently our main interest.

#### P02.069

##### A new entity: Report on two siblings with mental retardation

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Two siblings, 17-year-old female and 13-year-old male, born to consanguineous parents, were referred to the department of genetics because of having short stature, motor-mental retardation and complete absence of speech. On physical examination, both siblings had short stature, a prominent and hairy forehead, thick eyebrows, synophrys, acneiform scars on their faces, short and webbed neck, short hands and feet were present. Laboratory data including urine amino acid chromatography was normal, methylmalonicacid and dinitrophenylhydrazine, serum IGF-1 and IGF-BP3 levels were normal. Abdominal ultrasound was normal except mild splenomegaly. Skeletal survey was normal except coxa valga. Cranial MRI and echocardiography were not indicative for an abnormality. Cultured fibroblasts showed normal neuraminidase, beta hexozaminidase, beta galaktosidase activity. Enzymatic analyses of leucocytes a-iduronidase,  $\beta$ -glucuronidase,  $\beta$ -galactosidase,  $\alpha$ -fucosidase,  $\alpha$ -mannosidase, N-asetil glucosaminidase, glucosamine N-asetil transferase showed normal activity. Sialotransferrin pattern was normal and cases were not compatible with carbohydrate deficient glycoprotein syndrome. Karyotypes were normal. The siblings were not compatible with any identifiable genetic syndrome, and they could be an example of a new clinical picture.

#### P02.070

##### Etiology of Mental Retardation in Brazil: The first 200 institutionalized patients

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We have studied 200 mentally retarded (MR) patients from two Brazilians centers; 6% had mild, 48.5% moderate, and 37.5% severe MR. There were 114 male (57%) and 86 female(43%). Their ages varied from 6 month to 69 years, included 55.5% 0-15 years old, 42% 16 to 60 years old and, 2.5% > 60 years old.

It was impossible to perform the etiological diagnosis in 42 patients (21%); the environmental causes were established in 85 patients (42.5%); genetic diseases were diagnosed in 64 patients (32%). Chromosomal anomalies were detected in 28 patients (14%), including 24 cases of Down syndrome (12%) and 4 structural chromosomal anomalies. Genic abnormalities were detected in 29 patients (14.5%) with 6% (12 patients) presenting autosomal recessive, 0.5% ( 1 patient) with autosomal dominant, 1% (2 patients) with X-linked diseases, one of them (0.5%) affected by fragile X syndrome ; 1 patient (0.5%) with imprint defect (Beckwith-Wiedemann syndrome); and, we detected 13 families (6.5%) with pure mental retardation. We detected 8 patients (4%) of isolated CNS primary malformations and, 6 cases (3%) of inborn errors of metabolism ( 4 cases of phenylketonuria). The environmental causes (42 cases=21%) were: 33 patients (16.5%) with MR due to isquemic-hypoxic perinatal accident; 28 patients (14%) MR due to complications of prematurity; 11 patients (5.5%) MR due to prenatal infections and, 7 patients (3.5%) MR due to postnatal infections; and, 5 patients (2.5%) MR due to Fetal Alcohol syndrome.

#### P02.071

##### The clinical importance of the variability of tRNALys and its neighbouring mtDNA sequence

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**Background:** The mtDNA has a big variety, this proves the pathophysiological importance of the mtDNA-alterations. Pathogenic mtDNA mutations are frequently found in tRNA coding regions of the mitochondrial genome. The tRNA<sup>Leu</sup> gene is one of the hot-spots of the mtDNA. Many pathogenic mutations have been reported in tRNA<sup>Leu</sup> too.

**Design/Methods:** The most common mtDNA mutations were investigated in 470 patients, presumably with mitochondrial disease and in 100 controls with PCR-RFLP methods in the region of tRNA<sup>Leu</sup> and the intergenic region of COII and tRNA<sup>Leu</sup> genes. In patients, whose restriction patterns differed from the normal, bidirectional sequencing of this region was performed.

**Results:** The A8344G mutation was found in 8 cases. In one family (5 people) heteroplasmic substitutions T8312A and T8313G were

present. A heteroplasmic A8332G substitution was present in another family, and a homoplasmic A8347C substitution in 19 cases. We have demonstrated 5 polymorphisms and anthropologically markers (G8251A (n=19); G8269A (n=1); C8270T (n=4); G8292A (n=8); and a 9bp deletion (n=4). The 9bp deletion at the nt. 8271-8280 is an anthropologically marker of East-Asian origin. The substitution C8270T coexisted with this 9bp deletion in all cases.

Conclusion: We proved the enormous variability of the mt tRNA<sup>Lys</sup> and the hypervariable non-coding region between COII and tRNA<sup>Lys</sup>. The clinical symptoms developed as the result of one or more SNPs, or the synergistic effect of its combinations. On the basis of our investigations we suggest to start the screen for pathogenic mtDNA mutations in tRNA<sup>Lys</sup> and tRNA<sup>Leu</sup> genes.

#### P02.072

#### Metachromatic leukodystrophy, clinical course and molecular findings; A case report of an Iranian patient

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Metachromatic leukodystrophy or MLD is a rare autosomal recessive disorder of impaired breakdown of sulfatides that occur throughout the body, but are found in greatest abundance in nervous tissue, kidneys, and testes. The three clinical subtypes of MLD include late-infantile MLD, comprising 50-60% of cases; juvenile MLD, comprising about 20-30%; and adult MLD, comprising about 15-20%. Age of onset within a family is usually similar. All individuals eventually lose motor and intellectual functions. The disease course may be from three to ten or more years in the late infantile-onset form and up to 20 years or more in the juvenile- and adult-onset forms. Death most commonly results from pneumonia or other infection.

ARSA is the only gene associated with arylsulfatase A deficiency. MLD is suggested by arylsulfatase A enzyme activity in leukocytes that is less than 10% of normal controls using the Baum type assay. The ARSA gene which is located on chromosome 22q13, consists of eight exons encoding the 507 amino acid enzyme. Over 90 largely missense mutations and polymorphisms have been identified in the ARSA gene. The majority of mutations identified in patients with MLD are unique within individual families.

The identity of the mutation was confirmed by amplifying all eight exons by polymerase chain reaction which was followed by direct DNA sequencing. The individual described in our study showed a homozygous known missense mutation at c.1173C>G (p.T391S) in exon 7.

#### P02.073

#### Variable expression of the features of COFS Syndrome in two sibs

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Cerebro-oculo-facio-skeletal (COFS) syndrome is characterized by severe microcephaly, microphthalmia, blepharophimosis, arthrogryposis and characteristic dysmorphic face. It is a autosomal recessive degenerative disorder with prenatal onset. Although the syndrome has been described thirty years ago, its molecular basis has been recently described. Defective DNA repair and homozygous mutation in the ERCC6 gene underlies its pathogenesis.

We report on a family of unrelated parents who had two boys with variable expression of COFS syndrome. Both pregnancies were unremarkable, though reduced fetal growth has been noted. After the delivery both babies serious problems for adapting to the extra uterine life. Severity of the main signs of the syndrome was variable. Both had microcephaly under 3rd percentile. Facial dysmorphism included blepharophimosis, wide and nasal root, prominent philtrum, micrognathia, large and soft ears. The first sib had more evident microphthalmia especially of the left eye. This sib also had diastasis m. recti abdominis. Joint stiffness was present only in small joints of the hands and feet. The other sib had severely affected extremities with arthrogryposis in all joints. Ultrasonographic evaluation has been made in both of them, and MRI has been performed in the second, showing corpus callosum agenesis, cerebellar hypoplasia, reduced white matter of the brain. There are many reports of families with affected sibs having COFS syndrome. The present clinical signs of the sibs were variable, as are

in previously described family. Although molecular diagnosis of the syndrome has been elucidated, the reason for intrafamilial differences is still to be evaluated.

#### P02.074

#### Use of MLPA technique for the diagnosis of particular chromosomal abnormalities - clinical and genetic study of a case

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We present a case with multiple birth defects associated with mental retardation due to an abnormal karyotype in order to discuss the importance of MLPA in guiding the diagnosis.

Anamnestic data show that the infant is the only child of a young, unrelated, apparently healthy couple. She was born after an uneventful pregnancy, naturally, at 36 weeks, birth weight 2800g, height 48 cm, APGAR 8. Postnatal development evolved with severe failure to thrive and developmental delay.

Clinical examination of the child (1 year old) revealed: dysmorphic face (tall forehead, short palpebral fissures, deep set eyes, large mouth, large ears), short neck with excess of skin, skeletal abnormalities (congenital hip dysplasia, bilateral talus valgus, arthrogryposis), hypotonia and severe developmental delay.

Investigations: Echocardiography: patent ductus arteriosus, atrial septal defect; Neurological exam: generalized muscle hypotonia, arthrogryposis; Ophthalmologic exam: normal; Karyotype: 46 XX, add (9)(p24); Karyotype of the parents: normal; MLPA test (with P036C and P070 Mental Retardation Telomere Kit): triple dosage of probe DMRT1 from subtelomeric zone 9p (9p24.3). Add (9)(p24) is in fact dup9p. Comparison of the clinical features present in our patient and those in the literature for dup9p will be provided. In conclusion, we present a case with a particular chromosomal abnormality in order to illustrate a rare disorder and to discuss the use of MLPA in the identification of chromosomal abnormalities.

#### P02.075

#### Finding genetic causes of unexplained psychomotor retardation cases.

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Multiplex Ligation-dependent Probe Amplification (MLPA) is being routine used to detect subtelomeric alterations in patients with idiopathic mental retardation and other clinical features. FISH analysis, using telomere specific probes, is performed to confirm the aberrations identified by MLPA.

Here we present a family with three sons, two of them showing psychomotor retardation and language difficulty, but no dysmorphic features. Parents were non consanguineous and healthy. In all of them, karyotype was normal, at 550 level banding GTG. We performed MLPA analysis in order to detect possible subtelomeric rearrangements, and confirmed our results by FISH.

MLPA analysis were performed using the SALSA P069 Human Telomere containing one probe for each subtelomeric region from chromosome 1-22 and the two X/Y pseudoautosomal regions (MRC Holland, Amsterdam, The Netherlands). Fluorescence in situ hybridization (FISH) with subtelomeric probes (QBiogene) were carried out.

MLPA analysis showed evidence of a deletion in the terminal region of chromosome 3p (probe CHL1 gene in 3p26) and a duplication in the subtelomeric region 8q (probe KIAA0150 gene in 8.q24.3) in the two affected sons, and normal results in the other family members. FISH using probes 3pter (D3S4558) and 8qter (D8S595) confirmed the imbalanced subtelomeric rearrangements detected by MLPA in the affected sons and a balanced reciprocally translocation between 3p arm and 8q arm in the father. Mother and unaffected son were normal.

This study confirms that the combination of MLPA analysis and FISH allows to resolve cases, where the genetic causes of the clinical features would otherwise remain unexplained.

## P02.076

**Study of D2 dopamine receptor (DRD2) gene expression, in Morphine-sensitized mice in the absence and presence of LiCl**

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One of the consequences of repeated morphine administration is sensitization in which the intermittent exposure to a fixed dose of drug results in a greatly enhanced behavioral response to further morphine administration. The neurotransmitter, dopamine, is involved in several functions of the brain. Of all the five DRDs, D2 receptor has maximal affinity for dopamine; there is a growing evidence for the role of D2 receptor in different aspects of addiction.

In this study, the expression level of dopamine D2 long (D2L) and D2 short (D2S) isoforms of D2 receptor in morphine-sensitized mice were investigated in the absence and presence of LiCl (5 and 10 mg/kg). Morphine sensitization was induced by once daily injection of morphine (30 mg/kg) for 3 days, followed by 5 days wash-out. Using relative Real-Time PCR, D2L and D2S expression were examined in the brain regions including striatum, PFC and hippocampus. We found that morphine treatment leads to a significant increase in D2S level in the striatum and PFC but has no effect on D2L level in the examined regions. While administration of LiCl 5mg/kg along with morphine does not alter D2L and D2S isoforms, LiCl 10 mg/kg treatment results in a markedly increase in D2S but not D2L expression level in the striatum and PFC of sensitized mice. The result indicates that morphine sensitization leads to an increase in presynaptic D2S receptor expression level and is affected by lithium in a dose-dependent manner with low dose to inhibit and higher dose to enhance the effect.

## P02.077

**Mucopolysaccharidosis Type IH - A case report**

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Mucopolysaccharidosis Type IH (OMIM #607014) or Hurler Syndrome is the most common disorder of the group of seven mucopolysaccharide storage disorders. MPS IH is inherited in an autosomal recessive manner. The estimated incidence is approximately 1:100,000 newborns. MPS IH is caused by mutation in the gene encoding  $\alpha$ -L-iduronidase. Deficiency of  $\alpha$ -L-iduronidase leads to progressive accumulation of undegraded glycosaminoglycans in all bodily tissues. Our proband is a 5 years old male, the only child of an unrelated couple who was admitted in our clinic for severe respiratory distress. A comprehensive medical evaluation was made: prenatal and birth history, physical, neurologic and genetic examinations, biologic and imagistic evaluations (cardiac and abdominal ultrasonography, chest, hand and skull X-ray). By clinical examination we noticed: mental and motor development retardation, gibbus deformity, claw hand deformity, short stature, coarse facies, large head, prominent forehead, hypertrichosis, corneal clouding, hepatosplenomegaly, abdominal enlargement, inguinal hernia, wheezing. His past medical history revealed recurrent ear and respiratory infections. Positive diagnosis was based on association of clinical signs, imagistic evaluation and enzyme deficiency testing- which demonstrated absent  $\alpha$ -L-iduronidase activity in plasma. Unfortunately his parents refused enzyme replacement therapy (Al-durazyme®) in spite of his poor condition, knowing its evolution and prognosis. Proper genetic counselling was offered. Prognosis of our case is linked to the respiratory and cardiovascular complications. In conclusion, we present a case of MPS IH in order to illustrate this rare genetic disorder but also to discuss the positive diagnosis, the management and the genetic counseling.

## P02.078

**Severe congenital cyphoscoliosis associated with multiple congenital anomalies in an adolescent boy**

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Aim: To present a 17 yo boy, with multiple malformations admitted for an accurate treatment.

**Matherial and methods:** The patient was diagnosed at birth with congenital cyphoscoliosis, Fallot tetralogy, pulmonary atresia, single kidney and syndactyly of the left hand. His teeths, developed anarchic. At the age of 7 was operated for a Blalock Taussing shunt. He has 19.5 Kg, 132 cm, generalized muscular hypotonia and hypotrophia, cyanosis, congenital severe thoraco-lumbar sinistro-concave cyphoscoliosis, secondary sterno-condral asymmetry, apex located in left axillae, gr. III/6 continuous thoracic murmur, and is extremely clever. Laboratory tests revealed poliglobul and slightly elevated liver enzymes. He performed a lot of paraclinic investigations.

**Results:** The cardiac examination mentioned also: MAPCA, anomalies of the aortic arch and gr. II aortic insufficiency. Neurologically was found generalized muscular dystrophy. Abdominal ultrasound developed two hepatic tumors, one left kidney and multiple vascular anomalies. Surgical exam described a Marfan fenotype. Thorax Angio CT confirmed aortic arch anomalies, Lusoria artery and multiple arterial connections to the lungs. Abdominal MRI developed three hepatic tumors, one hepatic nodular fibrosis and two small hepatic hemangioma. The patient is in chronic heart failure treatment with Digoxin, antiaggregant therapy and vitamins for the liver. He is suspected of hemangioendothelioma. **Conclusions:** Because of his poor clinical condition, severe column cyphoscoliosis and multiple associated malformations, the patient was temporized for the final surgical cardiac repair. His column needs surgical stabilization. The hepatic tumors needs fine needle biopsy. We have to decide the priorities in treatment for a psedo-normal life.

## P02.079

**MURCS Association With Situs Inversus Totalis: A Case Report**

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MURCS association is a rare, developmental disorder which involves the Mullerian duct, the kidneys and the cervicothoracic spine. It is a sporadic disorder with unknown etiology. The prevalence is 1/50000 female. Occasionally, it may be accompanied by abnormalities involving various other organs or systems. A 24 year old woman came to our clinic suffering from primary amenorrhea, then she was diagnosed MURCS association.

Röntgenograms showed thoracolumbar vertebral defects-scoliosis and abdominal MRI revealed pelvic renal ectopy, the absence of the uterus and also the upper part (2/3) of the vagina. MRI revealed also presented situs inversus totalis which is a very rare anatomic condition. Her karyotype was normal 46,XX. This is the first report of situs inversus totalis in a case of MURCS association.

## P02.080

**Two cases of Myhre syndrome with retinopathy: further delineation of the phenotype**

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Myhre syndrome is characterised by facial dysmorphism, short stature, joint limitations, brachydactyly, muscle hypertrophy, hearing loss and mental retardation. Specific radiological findings include thickened calvarium, hypoplastic iliac wings, broad ribs, and abnormal vertebrae. No genetic basis has been identified.

We report two unrelated patients with Myhre syndrome, who both had retinopathy, undescribed up to date.

Patient 1, a 13 year old male, presented with typical dysmorphism, language delay, learning difficulties, obesity, precocious puberty, brachydactyly, athletic build, joint limitations, and thick skin. Height was nor-

mal with advanced bone age. Mild unilateral transmission hearing loss was detected. Strabismus was noted and electroretinogram showed macular retinopathy.

Patient 2, a 10 year old female, presented similar clinical features as patient 1 leading to a diagnosis of Myhre syndrome. She had intra uterine growth retardation and then showed normal stature with advanced bone age. She presented mild transmission hearing loss. Unilateral cataract was diagnosed at 6 months. Severe myopia was noted and electroretinogram showed macular retinopathy.

Karyotype banding, subtelomeric analysis, and metabolic screening were normal, and CGH array analysis is underway for both patients. Description of retinopathy in our patients suggests that electroretinogram should be systematically undertaken in Myhre patients, in order to confirm this new feature.

At this time, the main differential diagnoses of Myhre syndrome are mainly bone disorders (such as geleophysic and acromicric dysplasia). However, the association of bone, muscular, auditory and retinal involvement could suggest that a metabolic disorder, such as mitochondrial dysfunction, could be responsible for Myhre syndrome.

#### P02.081

#### Pancreatic hypoplasia presenting with neonatal diabetes mellitus in association with congenital heart disease and developmental delay

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Congenital pancreatic hypoplasia is a rare cause of neonatal diabetes. We report a case series with pancreatic agenesis and congenital heart disease. Case (1) was born prematurely at 34+2 weeks gestation and developed hyperglycemia within the first 12 hours of life requiring insulin. She also has exocrine pancreatic insufficiency needing supplementation. Cardiac imaging revealed ventricular septal defect, patent ductus arteriosus and pulmonary artery stenosis. She is severely developmentally delayed and has abnormalities on cranial imaging (focal microcalcification, gliosis and cerebral atrophy).

Case (2) is a child who had antenatally detected truncus arteriosus who developed diabetes in the neonatal period post surgery. Imaging of the abdomen confirmed pancreatic agenesis. He has microcephaly and is developmentally delayed.

Case (3) is a female infant born at 34 weeks gestation with an antenatal diagnosis of Tetralogy of Fallot. She manifested hyperglycemia and pancreatic insufficiency from day 3 of life requiring replacement therapy. Repeated imaging failed to visualise the pancreas. She had initial motor delay, but is currently well.

Investigations included sequencing of GCK, ABCC8, IPF1, SUR1, NEUROD1, GATA4, PTF1A and KCNJ11 genes, but no mutation was found. Genetic investigation to exclude paternal UPD 6, methylation aberrations and duplications of 6q24 was also negative. Array CGH in case (1) showed a paternally inherited ~250 kb dup(12)(q24.33), not present in the others.

Permanent neonatal diabetes mellitus due to pancreatic hypoplasia with congenital heart disease has been reported before and may represent a distinct condition (Gurson CT et al 1970; Yorifuji T et al 1994).

#### P02.082

#### Severe combined immunodeficiency, microcephaly and failure to thrive. A chromosomal breakage syndrome with mutations in the NHEJ gene

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Recently mutations in the *NHEJ* (nonhomologous end-joining factor) gene have been described (Ahnesorg et al., 2006; Buck et al., 2006) causative for a new chromosomal breakage syndrome characterized by severe combined immunodeficiency, microcephaly, developmental delay and subtle facial dysmorphism.

Here we present the clinical, cellular and molecular findings in a Turkish boy, first son of consanguineous parents. At term, he showed low birth measurements [weight 2290g (-4.2 SD), length 49 cm (-2 SD), OFC 32 cm (-4 SD)]. He developed respiratory infections and a severe autoimmune anemia. At age 13 months he had a weight of 5200g (-3.3 SD), height: 64 cm (-2.8 SD) and OFC: 38.5 cm (-5.3 SD). Motor and mental developmental development was nearly normal. At the age of 4 months a tentative clinical diagnosis of Nijmegen breakage syndrome was made. Chromosomal breakage analysis revealed a high rate of chromosomal breakage and extremely high levels of damage after irradiation. Molecular testing showed a known homozygous mutation (R178X) in exon 5 of the *NHEJ* gene.

We describe the clinical, cellular and molecular findings of this new autosomal recessive chromosome breakage syndrome with respect to the literature.

#### P02.083

#### Broad first digits, facial and oral anomalies, and developmental delay in a three-generation family: A Novel Syndrome?

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A characteristic combination of facial, oral, and digital anomalies was observed in a three-generation Caucasian family, with a segregation pattern suggestive of an autosomal dominant inheritance. The facial dysmorphism included prominent forehead, almond shaped eyes with upslanting palpebral fissures and medial epicanthal folds, broad nasal bridge, thin upper lip, and posteriorly rotated ears. Observed oral anomalies included high arched palate, torus palatinus and midline groove of the lower lip. All affected family members had broad thumbs and great toes, speech delay, and mild mental retardation. Seizures, macrocephaly, abnormal brain MRI, somatic overgrowth, congenital heart defects (VSD with coarctation of the aorta), and cryptorchidism were also observed among various members of this family. Microsatellite analysis excluded linkage to CREBBP and EP300, the genes associated with Rubinstein-Taybi syndrome. Additionally, Simpson-Golabi-Behmel syndrome was considered, but X-linked inheritance was excluded by the presence of male-to-male transmissions and two affected females. Cytogenetic studies, including karyotype, subtelomere screen, and FISH for 22q11 deletion syndrome, were normal. Taken together, we believe that this condition represents a novel dysmorphic genetic syndrome.

#### P02.084

#### Xp11.4 deletion identified by array-CGH in a mother and her daughters causing OFCD phenotype

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We report on a familial case of syndromic bilateral congenital cataract with dysmorphic features in a mother and both of her daughters. The younger daughter, referred to the genetic clinic, showed additional malformations comprising atrial septal defect, facial dysmorphic features (synophrys, high nasal bridge, small cup shaped ears, bifid uvula, long philtrum, microstomia), dental anomalies (delayed eruption, oligodontia), skeletal manifestations (irregular metacarpal epiphyses ossification) and moderate developmental delay. High resolution karyotype was normal. The array-CGH analysis identified an Xp11.4 deletion of

2-2.2 Mb. This deletion, which encompasses several genes including *RPGK*, *OTC*, *TSPAN7* and *BCOR*, was also present in the mother and the sister of the proband. Both of them also have congenital cataract and dental anomalies, and hammer toes as well as bilateral 2-3 toe syndactyly were also present in the sister.

Among the deleted genes, *TSPAN7* plays a role in mental retardation, and *BCOR* (BCL6 co-repressor) seems to be the best candidate gene to explain the familial clinical features. Several frameshift, deletion, nonsense mutations and exonic deletions were found in *BCOR* causing Oculo-facio-cardio-dental (OFCD) syndrome. OFCD syndrome is an X-linked dominant condition with presumed male lethality characterized by multiple congenital anomalies: cardiac abnormalities (septal defects), ocular malformations (congenital cataracts, microphthalmia), facial dysmorphic features, cleft palate and dental anomalies (delayed dentition, oligodontia, abnormal shaped teeth, radiculomegaly). Some reported cases developed psychomotor and mental retardation.

#### P02.085

#### Renal insufficiency, a frequent complication of oral-facial-digital syndrome type I

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The oral-facial-digital syndrome type I (OFDI) involves multiple congenital malformations of the face, oral cavity and digits. It is the most frequent type of oral-facial-digital syndrome, characterised by X-linked dominant mode of inheritance with lethality in males. The *OFD1* gene encodes for a centrosomal protein located both in the primary cilium and in the nucleus, leading to consider the OFDI syndrome as a ciliopathy. A polycystic kidney disease (PKD) is reported in almost one third of OFDI patients, but long-term outcome of the renal disease has been essentially described through isolated cases. We report on the renal manifestations of a cohort of 34 patients with an identified mutation in the *OFD1* gene. Patients are aged of 1 to 68 years. Their median follow-up is of 16.5 years. Among them, 12 (35%) patients presented with PKD (11/16 (69%) if only adults were considered) with a median age at discovery of 29 years. Ten of them also presented with renal impairment and six were grafted. One grafted patient under immunosuppressive treatment died from a malignant tumor originated from a native kidney. In patients aged 36 or more, the probability to develop renal failure was estimated to be more than 50%. Besides, neither genotype-phenotype correlation nor clinical predictive association with renal failure could be evidenced. These data reveal an unsuspected high frequency with age of renal impairment in OFDI syndrome. Systematic ultrasound scans and renal function follow-up are therefore highly recommended for all OFDI patients.

#### P02.086

#### Mutational spectrum of the Oral-facial-digital type I syndrome: a study on a large collection of patients

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Oral-facial-digital type I (OFDI; MIM 311200) syndrome is a male lethal X-linked dominant developmental disorder belonging to the heterogeneous group of Oral-facial-digital syndromes (OFDS). OFD type I is characterized by malformations of the face, oral cavity and digits. CNS abnormalities and cystic kidney disease can also be part of this condition. This rare genetic disorder is due to mutations in the *OFD1* gene that encodes a centrosome/basal body protein necessary for primary cilium assembly and for left-right axis determination, thus ascribing OFDI to the growing number of disorders associated to ciliary dysfunction. We now report a mutation analysis study in a cohort of 109 unrelated affected individuals collected worldwide. Putative disease-causing mutations were identified in about 80% of patients. We describe 67 different mutations, 64 of which are novel, including 36 frameshift, 9 missense, 11 splice-site and 11 nonsense mutations. Most of them concentrate in exons 3, 8, 9, 12, 13 and 16, suggesting that these may represent mutational hotspots. Phenotypic characterization of the patients collected provided a better definition of the clinical features of OFD type I. Differently to what previously observed, our results indicate that renal cystic disease is present in 60% of cases with over 18 years of age. Genotype-phenotype correlation reveal significant associations of the high-arched/cleft palate most frequently associated to missense and splice-site mutations. Our results contribute to expand our knowledge on the molecular basis of OFD type I syndrome. In addition these results will help in defining the clinical spectrum and recognition of Oral-facial-digital syndromes.

#### P02.087

#### Isolated oligodontia and hypodontia in multiplex families

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**Introduction:** Oligodontia is defined as congenital lack of more than six teeth and hypodontia is used when one to six teeth are missing. Oligodontia is a rare anomaly of tooth number while hypodontia is a common one. Both anomalies have a genetic background and are inherited in successive generations of a family.

**Objectives:** to evaluate and compare the pattern of missing teeth in families, to observe similarities and differences of dental phenotype among affected relatives, to characterize the mode of inheritance and to identify distinct groups of patients for further molecular investigations.

**Patients and Methods:** Clinical examinations were carried out on 6 Caucasian patients and their affected first-degree relatives from 3 families with a family history of missing permanent teeth. Combined examination of clinical phenotypes and orthopantomograms improved the precision of diagnosis. Family study was used to determine whether there is a hereditary basis for oligodontia or hypodontia.

**Results:** We describe tooth agenesis in three main groups: mother-daughter, sister-sister and brother-sister. None of the patients and their first-degree relatives shared similar patterns of missing teeth. No correlation exists between the patterns of missing permanent teeth in related individuals. Predominant dental phenotype involved anterior teeth agenesis and symmetrical (left - right) hypodontia. Anomalies of tooth-size and tooth-shape were also observed in association with hypodontia but not with oligodontia. Gender difference did not influence the severity of phenotype.

**Conclusions:** Multiplex family research allows for the study of inheritable oligodontia/hypodontia, pattern of inheritance and genetic cause.

#### P02.088

#### Otopalatodigital syndrome type 2, perinatal approach and identification of a new mutation in the Filamin A gene

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**Introduction:** Otopalatodigital syndrome type 2 (OPD2) is part of the so-called spectrum otopalatodigital disorders, it presents an X linked mode of inheritance and males generally have a more severe phenotype than women. Mutations responsible for the phenotype are found in the *FLNA* gene located in Xq28, the Filamin A protein is widely expressed and has an essential role in migration and cell morphology.

**Case Report:** Newborn mexican male patient, first pregnancy of healthy and non-consanguineous parents. Prenatal ultrasound as-

essment was carried out at 23.3 weeks of gestation, the study revealed intrauterine growth restriction, ribs with angle grinding, cleft hands and bowed femurs. An amniocentesis was performed showing a normal 46, XY karyotype. Physical examination showed wide fontanels and sutures, broad face, downslanting palpebral fissures, broad and depressed nasal bridge, anteverted nares, central cleft palate, microretrognathia and rotated and low implanted ears. Short neck, hands with digitalization of thumbs, diastasis between first and second finger bilaterally, clinodactyly of bilateral fifth fingers and cutaneous syndactyly between third and fourth fingers with hypoplastic, convex nails. X-rays of the tubular bones showed bowed humerus, femur and tibia, both fibulas were small.

**Results:** Molecular analysis of exon 3 of *FLNA* gene showed an insertion at codon 209 that generates a frameshift without causing a premature stop codon in the patient and his mother and has not been previously reported.

**Discussion:** A clinical diagnosis of OPD2 was initially suspected in our patient based on prenatal and postnatal clinical findings. Clinical evolution and molecular studies confirmed the disease.

#### P02.089

#### Spectrum of oral clefts in the light of contemporary research study

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**Aim.** To apply contemporary knowledge in genomics, modern techniques for experimental testing and data analysis in the investigation of genetic and genomic basis of oral clefts (OCs) and the acquired knowledge introduced into the practise of Lithuanian health system. **Material and method.** The study group consisted of 250 patients with OCs and one or more major congenital anomalies. According to syndromologic, cytogenetic and molecular genetic data analysis were categorized into two groups: recognized conditions (40.8%, 102 cases) and unknown origin (59.2 %, 148 cases) during the past decade. The type of cleft was classified according to ICD-10 and LAHSHAL classifications.

**Results.** 74 patients had recognized syndromes and sequences (20 different units). One of the most common was Pierre Robin sequence (32 cases), 6 cases of holoprosencephaly, 5 cases of OFD syndrome, 4 of amniotic band sequences. Among the cases with recognized conditions 28 had chromosomal abnormalities: such as trisomy 13 (16 cases), trisomy 21 (1 case) and trisomy 18 (1 case), others were partial trisomies (46, XY, rec(14) dup(14) inv(14)(p11.2;q32.1); 46, XX, der(13)t(13;20)(p11.2;p13)), partial deletions (46,XX, del(18)(q21.32;pter)), reciprocal translocations (46, XX, t(10;11)(p11.2;q23.3), 46, XX, t(2;6)(p21;p25), 46, XX, t(8;16)(p21.1;p13.1)) and two mosaic karyotypes (46, XY [1]/46, XY, r(21) [10]/46, XY, -21, +mar [30]/45, XY, -21 [9] and 47,XX,+mar [17]/46,XX [3]). DNA microarray based on APEX2 technology with 640 SNPs in 43 CLP candidate genes was carried out for 104 triads.

**Conclusion.** The obtaining results were relevant to further prophylaxis of the definite population.

#### P02.090

#### Search for genomic imbalances in a cohort of 20 patients with oral-facial-digital syndromes negative for mutations and large rearrangements in the *OFD1* gene

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Oral-facial-digital syndromes (OFDS) result from the association of abnormal clinical features affecting almost invariably the face, mouth and digits. Other organ systems can be involved, defining a complex nosology including 13 specific subtypes. Some of them have been described in only one or two reports. Some overlap between subtypes is suspected. The clarification of this complex nosology could only come from a better understanding of their molecular bases. However, only the *OFD1* gene responsible for OFD type I is currently known. Because of the presence of multiple congenital abnormalities (MCA) and/or mental retardation in some OFDS, we questioned about the possibility of submicroscopic rearrangements as a cause of OFDS. We therefore performed high-resolution array-CGH (244 or 105K Agilent) in a series of 20 OFDS negative for mutations and large rearrangements in the *OFD1* gene, gathered through an international collaboration. Only seven patients could be assigned to a specific subtype of OFDS. Three patients had malformations that are not commonly found in the different OFDS subtypes, including gyration abnormalities, abnormal lung lobulation, deafness, imperforate anus, and trigonocephaly. Nine patients had mental retardation. We failed to evidence submicroscopic chromosomal imbalances in this series. These results suggest that chromosome imbalances appear rarely involved in OFDS and that mutations within single genes may be responsible for these phenotypes. Further work at a molecular level is needed to identify the causes and allow appropriate counselling for families regarding specific recurrence risk and prognosis.

#### P02.091

#### Confirmatory report of Megarbane autosomal recessive oto-facial syndrome

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In 2005, Megarbane et al reported two sisters from a Lebanese consanguineous family (their parents were first cousins), with a new oto-facial syndrome. These patients presented microcephaly, dysmorphic features, very dysplastic low-set ears, malformation of the middle ear and short stature. In addition, one of the patients had a posterior cleft palate, and the other an oesophageal atresia. Because of the recurrence in sibs and the parental consanguinity an autosomal recessive mode of inheritance was suggested.

We report a similar observation concerning an 18-year-old boy. This patient was the second of three children from non consanguineous parents. There was no remarkable familial history. He presented a mandibulo-facial dysostosis with microcephaly, extremely rudimentary and low-set helix of ears, absence of the external auditory channel, long nose with low columella, short philtrum, everted lower lip and small chin. A low implantation of the thumbs, with ankylosis of the right one, was noticed. The patient also presented a mild mental retardation and severe conduction deafness. The CT scan revealed hypoplastic middle ear cavity with absence of aeration and underdeveloped auditory ossicles. At the age of 18, the microcephaly persisted (-3 SD), stature was normal (171cm) but stayed inferior to the familial height. Chromosomal investigations (standard karyotype and array-CGH) were normal. The molecular screening of the CHD7 gene responsible of the CHARGE association was negative. We compare this observation with other known oto-facial syndromes and especially the familial observation of Megarbane.

#### P02.092

#### Phenotypic characterization of PHACE(S) Association, first Italian study based on Eight patients

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PHACE (OMIM No. 606519) is a neurocutaneous syndrome encompassing: Posterior cranial fossa malformations (PCFM), Facial Hemangiomas (FH), Arterial anomalies (AA), Cardiac Anomalies (CA), and Eye Abnormalities (EA). PHACE(S) is used when ventral developmental defects are present. The underlying pathogenesis of PHACE is unknown. To date less than 300 patients are reported world wide since initial description.

Aims of our study:

- Describe the phenotype in a patients' series.
- Verify already present data in literature.
- Obtain diagnostic standards.
- Obtain useful information from prognostic and therapeutic points of view.
- Identify new etiopathogenetic hypotheses or validate already present ones in literature. This is a multidisciplinary study of 8 patients referred to our Tertiary Medical Center.

The diagnosis of PHACE was established if the characteristic large, plaque-like, or segmental FH were observed associated to any of previously described anomalies. According to our protocol all patients undergo vascular surgeon, cardiologist, pediatrician, geneticist, ophthalmologist, neuroradiologist and whenever indicated cardiosurgeon specialist visits. Blood sample is performed for standard Karyotyping and Array CGH. Informed consent was obtained from the parents of each participating child.

Our preliminary results confirmed the strong female predominance (6/8) and absence of familial cases.

Although PCFM constitute the most commonly observed developmental brain abnormalities in PHACE, none of our patients had any. On the other hand, 6 patients presented cerebrovascular abnormalities. AA, mostly aortic coarctation, was present in 6 and none had CA. EA are present in 4 patients. We present the classification of most frequently observed malformations, Array-CGH results and different therapeutic aspects.

#### P02.093

##### Polycystic kidney disease associated with 21-hydroxylase deficiency - case report

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Background: PKD in children is defined as an inherited disorder involving progressive cystic dilations at any point along the nephron and varying degrees of extrarenal involvement (gastrointestinal tract, cardiovascular system, reproductive system, CNS), and is known now as a ciliopathy disease. The issue of this paper is to present a case of PKD associating 21-hydroxylase deficiency, diagnosed and followed-up in our clinic since newborn. Method: female girl, aged 3 years and 4 month, presented as newborn with clitoris hypertrophy, SGA. Endocrine evaluation (17-hydroxiprogesterone, cortisole, testosterone, DHEA) established the diagnosis of adrenogenital syndrome - 21-hydroxylase deficiency. Elevated serum BUN and creatinine led to a nephrologic imagistic evaluation, which showed a polycystic right kidney and a hypoplasia of the left kidney, CrCl = 12,12 ml/min, no hypertension. Substitutive treatment with hydrocortisone orally was initiated. Close follow-up included: growth parameters, clinical examination, renal function, Ca - P metabolism, hematologic evaluation, hormone determinations, imagistic assessment. Results: The patient developed progressive renal failure (CrCl = 16,3 ml/min), anemia (Epoetin started december 2007), renal osteodystrophy (1,25-dihydroxy vitamin D3 administration), presented few episodes of urinary tract infections and failure to thrive. Clinical evaluation of the genitalia and hormone determinations showed a good response to substitutive treatment. The evaluation of growth hormone therapy, started december 2007, is in course. Conclusion: metabolic balance was difficult to obtain because of the associated pathology, and the 21-hydroxylase deficiency could be an unfavorable factor for the evolution of the renal disease.

#### P02.094

##### Bilateral vocal cord paralysis, left lower motoneurone 7<sup>th</sup> cranial nerve palsy and left 12<sup>th</sup> cranial nerve palsy - another case of Plott syndrome?

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Plott syndrome was first described in 1964 by Plott et al. in three brothers with permanent laryngeal abductor paralysis and mental retardation [Plott et al., 1964]. One affected male had a left 6<sup>th</sup> cranial nerve palsy. In 1973, Watters and Fitch described a pedigree which made X-linked recessive inheritance likely [Watters et al., 1973]. Two brothers were affected as well as a first cousin once removed through females. The mental retardation seen in two of these three patients was more severe than in the previously reported patients. We report a male patient who presented at birth with stridor. He required a tracheostomy and bilateral vocal cord paralysis was diagnosed. Neurological examination revealed a left lower motoneurone 7<sup>th</sup> cranial nerve palsy and a left 12<sup>th</sup> cranial nerve palsy. He showed mild motor delay and walked at 22 months. He is the only affected child in the family, and therefore no definite conclusions can be drawn as to possible inheritance. Autosomal recessive inheritance of bilateral vocal cord paralysis has also been postulated in the literature [Koppel et al., 1996], as well as autosomal dominant inheritance [Schinzel et al., 1990; Manaligod et al., 1998], but in these patients, no developmental delay was observed, nor was there involvement of any other cranial nerves. Plott syndrome therefore seems to be the most likely diagnosis in our patient.

#### P02.095

##### Poland Syndrome - case report

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Poland Syndrome (PS) is named after Alfred Poland, who described it in 1841. Poland described a range of several abnormalities, among which the absence of the pectoralis major and pectoralis minor muscles and syndactyly. The incidence of PS was estimated at 1 in 30,000. The syndrome is thought to be of vascular origin. Our female patient was first - born child. This was controlled and non-complicated pregnancy of not consanguineous parents. The baby was born on term. Growth parameters were weight=2620 g; length=46 cm. Mother noticed smaller right hand and right nipple and asymmetry of thorax at birth. Development milestones have been appropriate for her chronological age. She attained menarche at age 14. Investigation of family history revealed a deaf and mute grandfather. The proband was first observed by geneticist at age 14. Growth parameters were weight=43 kg; height=153 cm; and head circumference=53 cm (below 10th percentile). Vital parameters were normal. At the physical examination there were aplastic right breast and hypoplastic right nipple. In addition, she lacked the sternal origin of the right pectoralis muscle and axillary hair. She had shortening of right upper limb, hypoplastic right hand and nails with brachydactyly of all fingers and clyndactyly of 5th. Lower limbs were normal and symmetric. Karyotype revealed 46,XX. Radiographic examination showed shortening of middle phalanxes of the right hand and lack of soft tissue shadow of right breast. Treatment should include reconstructive surgery and possibly implantation of bio-engineered tissue in adult period.

#### P02.096

##### Poland-Möbius syndrome associated with celiac disease-case report

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Poland-Möbius syndrome associates two rare congenital syndromes and consists of craniofacial, musculoskeletal, cardiovascular defects and cranial nerve palsies.

We report a case of a male newborn with Poland-Möbius syndrome,

who was admitted in the IIIrd Pediatric Clinic Timisoara. The patient, born at 38 week of gestation, with 3000g, was the second child of young, healthy and unrelated parents. The older brother has no anomalies. There was no history of maternal irradiation or drug ingestion during pregnancy, and no family history of any congenital birth anomalies.

Clinical examination revealed underdevelopment of the left pectoralis major muscle, absence of the left areola, absence of the distal phalange of the left index, dextrocardia (confirmed by chest radiography, electrocardiography and echocardiography). He has also gastroesophageal reflux, confirmed by Barium Swallow X-ray. Abdominal sonography was normal. Neurologic examination revealed generalized hypotonia, convergent strabismus, unexpressive face, right facial palsy and swallowing difficulties. Chromosomal analysis was normal.

The patient was admitted several times in our clinic, presenting respiratory infections with respiratory insufficiency. In time, he developed signs of severe malnutrition, so that malabsorption tests were performed. We found positive IgA/IgG anti-tissue transglutaminase antibodies (IgA/IgG tTG), as well as Immunoglobulin A anti-endomysium antibodies (EMA) serum levels.

We performed enteroscopy with jejunal biopsy, which revealed Marsh IIIC villous atrophy. Haplotype analysis showed DQ2 in cis conformation (DQA1\*0501 and DQB1\*0201 alleles).

Conclusion. We present a rare association between Poland-Möbius syndrome and celiac disease. It is unclear if their etiology is common, or there are just two coincidental factors.

#### P02.097

##### Parkinsonism, axonal neuropathy and cerebellar signs in two siblings with recessive POLG mutations

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Mutations in POLG, which encodes the catalytic subunit of mitochondrial DNA polymerase gamma, have been identified in patients with diverse phenotypes. For example, recessive mutations are found in Alpers syndrome, autosomal recessive progressive external ophthalmoplegia (rPEO) and ataxia neuropathy spectrum (ANS) whereas dominant mutations are found in families with autosomal dominant PEO (adPEO) in which there may also be parkinsonism and premature ovarian failure. In 2006, two sisters with early onset parkinsonism and neuropathy, but without PEO, were found by Davidzon and colleagues to be compound heterozygotes for the recessive POLG mutations G737R and R853W.

We report a brother and sister with the same two mutations. The sister presented with parkinsonism aged 28 years and subsequently developed dysarthria. The brother was ataxic from age 18 years and later developed dysarthria and nystagmus. Ophthalmoplegia and ptosis were absent, however both had a severe, axonal, predominantly sensory neuropathy. MRI revealed no intracranial abnormality but prominence of the temporalis muscle was noted in both. Muscle biopsies were not done. The heterozygous parents were clinically normal. These siblings are remarkable firstly because parkinsonism is only rarely associated with recessive POLG mutations and secondly because patients with ANS due to POLG mutations usually have typical MRI abnormalities.

#### P02.098

##### Mutations in Human Beta-2b Tubulin Result in Asymmetrical Polymicrogyria

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Polymicrogyria is a relatively common but poorly understood defect of cortical development characterized by numerous small gyri and a thick disorganized cortical plate lacking normal lamination. We show an association between bilateral asymmetrical polymicrogyria and de novo mutations in a beta-tubulin gene, TUBB2B, in four patients and a 27 GW(gestational week) fetus. Neuropathological examination of the fetus revealed an absence of cortical lamination associated with the presence of heterotopic neuronal cells in the white matter, and neuronal overmigration through breaches in the pial basement membrane, reminiscent of "cobblestone-like" phenotypes observed in a Gpr56-/- mice model. In utero RNAi-based inactivation demonstrates that TUBB2B is a new gene that is critically involved in neuronal migration. We also show that two disease associated mutations lead to an impaired formation of tubulin heterodimers as a result of deficiencies in the complex chaperone-dependent tubulin heterodimerization pathway. These observations, together with previous data, demonstrate that disruption of microtubule-based processes underlies a large spectrum of neuronal migration disorders that includes not only lissencephaly/pachygryria, but also polymicrogyria malformations.

#### P02.099

##### Is the pattern of root resorption in human teeth dependent on the gene amelogenin?

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Introduction. It has been demonstrated that abnormal resorption of primary roots is associated with unexpected resorption of permanent roots (Bille et al., Eur J Orthod 2008; 30(4): 346-51). This leads to the hypothesis that root resorption is not always acquired, but can be inherited. This preliminary study is an immunohistochemical study on human teeth focusing on the gene amelogenin. Amelogenin has formally been associated with root resorption in mice (Hatekeyama et al., J Biol Chem 2003; 278(37): 35743-8).

Material. 18 primary teeth extracted from 10 children due to dental treatment.

Method. Paraffin sections for immunohistochemistry reaction were pre-treated with Tris-EDTA pH 9 at 60°C for 90 minutes. Sections were washed in TBS, encircled with a Dako pen (Dako, S2002), washed in TBS. Sections were incubated with Peroxidase-Blocking Solution (Dako, S2023), washed in TBS, incubated in primary antibody for 60 minutes using: Anti-Amelogenin (ABIN 187765, antibodies-online.com) diluted 1:3000 (Antibody Diluent, Dako, S2022).

Sections were washed in TBS, incubated with peroxidase labelled polymer (Dako, K5007), washed, incubated in Substrate Buffer/DAB+ Chromagen (Dako, K5007), washed in distilled water, counter stained in Carazzi's haematoxylin (Dako, S3301), washed, dehydrated and coverslipped using Pertex (Histolab, Sweden).

Results. Strong amelogenin expression was seen in the periodontal membrane as small and well distinct islands in regions without root resorption, while amelogenin expression was weak and mostly absent in resorption lacunas.

Conclusion. The present study showed that amelogenin might play a decisive role in human root resorption and therefore might influence an inherited pattern of root resorption.

#### P02.100

##### Restrictive dermopathy in a newborn caused by null mutation in ZMPSTE24 gene

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We described a 4 days boy old with tight, translucent skin, prominent vessels, skin erosions and dysmorphic findings consisting of hypertelorism, antimongoloid axis, sparse eyelashes and eyebrows, pinched nose, natal teeth, microretrognathia and 'o' shaped mouth. Multiple

joint contractures, dysplastic clavicles and thin ribs were also noteable. He died on 14 days of his life from respiratory distress. The patient was diagnosed as Restrictive Dermopathy which is a rare, lethal autosomal recessive disorder characterized by; tight and rigid skin with erosions, prominent superficial vasculature and epidermal hyperkeratosis, small mouth, small pinched nose, micrognathia, sparse or absent eyelashes and eyebrows and joint contractures. Restrictive dermatopathy is caused by the mutations in the zinc metalloproteinase gene ZMPSTE24 or LMNA. Analysis by direct sequencing of the gene ZMPSTE24 was performed and a single base insertion on exon 9 was identified in the patient on homozygous state (c.1085\_1086insT) leading a null mutation (p.Leu362PhefsX19). The parents latter demanded genetic consultation for next pregnancy. Found mutation on ZMPSTE24 was searched on the DNA derived from chorionic villi specimen and seen that the fetus inherited normal alleles from both of the parents.

#### P02.101

##### Restrictive dermatopathy in two siblings caused by novel compound heterozygous mutations of the ZMPSTE24 gene

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Restrictive dermatopathy (RD) is a rare, lethal disorder caused by mutations in the ZMPSTE24 gene (autosomal recessive) or in the LMNA gene (autosomal dominant). To date, about sixty cases of RD have been described. The signs of RD are very characteristic and include intrauterine growth retardation, thin, tight skin, superficial vessels, typical face changes and joint contractures. Children die within the first week of life. We observed the recurrence of the disease in a Polish family and report the identification of two novel inactivating ZMPSTE24 mutations. The children showed respectively, during the prenatal period: IUGR, decreased fetal movements, polyhydramnios, for the first child, and normal pregnancy to 30 weeks of gestation for the second. At premature delivery (32 and 33 weeks of gestation) both children showed typical facial features: hypertelorism, down-slanting palpebral fissures, pinched nose, posterior rotated ears, micrognathia, mouth in "o" position, skin and skeletal anomalies: thin and rigid skin with erosions and scaling, prominent superficial vessels, multiple joints contractures, camptodactyly, absent and small nails, rocker-bottom feet and narrow chest. Biological material was available for the second child: skin histology revealed thinned epidermal layers, focal hyperkeratosis, partial parakeratosis. Hair follicles and sebaceous glands were immature and poorly developed. The dermis showed absence of elastic fibers. Molecular analyses could be performed on the second child and his parents. Two novel, compound heterozygous, inactivating mutations of the ZMPSTE24 gene were observed in exon 1 (c.50delA; p.K17SfsX21) and 5 (c.584\_585delAT; p.Y195Ffs22X). The autosomal recessive inheritance was confirmed by the parents' molecular analysis.

#### P02.102

##### SC phocomelia/Roberts Syndrome Spectrum - A Case Report of an Adult with Review of the Literature

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Roberts syndrome (RBS) (OMIM #268300) is a rare autosomal recessive disorder characterized by tetraphocomelia (symmetrical limb reduction), craniofacial anomalies, growth retardation, mental retardation, cardiac and renal abnormalities. Karyotype investigations in affected patients characteristically reveal premature centromere separation, or heterochromatin repulsion. The syndrome is caused by mutations in the ESCO2 gene, which is located at locus 8p21.1, and

encodes a protein essential in establishing sister chromatid cohesion during S phase. Allelic to this condition is SC phocomelia (SC) (OMIM #269000), which has a milder phenotype compared to RBS with less severe symmetric limb reduction, flexion contractures of various joints, minor facial anomalies, growth retardation and occasionally, mental retardation. Individuals with SC typically survive to adulthood whereas severely affected RBS infants may be stillborn or die in the post-natal period. As a result, there is little literature about the follow-up of adults with the spectrum of SC phocomelia/Roberts syndrome or the recommended management. We report an adult presentation of SC phocomelia/Roberts spectrum disorder with a history of major cardiac malformation in childhood, mild facial anomalies, normal intelligence and premature centromere separation. A literature review is presented focussing on adult manifestations of this condition. Finally, we establish follow-up guidelines based on the reviewed cases.

#### P02.103

##### Is Schinzel-Giedion syndrome a genomic disorder?

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Schinzel-Giedion syndrome (SGS) is a severe neuro-developmental disorder characterized by distinctive face, short stature, limb abnormalities, sclerosis of the basis of the skull, wormian bones, gap in occipital skull, broad ribs, curved long bones and hypoplastic phalanges. Hydronephrosis, congenital heart defects and urogenital anomalies have been described. Inheritance is considered autosomal recessive. A boy was observed in 1997 after birth for short stature, hydronephrosis, mild pulmonic stenosis, unilateral cryptorchidism and limbs abnormalities. He developed obstructive apnoeas and swallowing difficulties. At the age of 18 months he was still short with hypotonia, pyramidal signs, epilepsy with multifocal EEG anomalies, no eye contact, high myopia, nasolacrimal duct stenosis, a large fontanel, high forehead, hypertelorism, midface hypoplasia, arched eyebrows with synophrys, chubby cheeks, broad nose, large mouth and protruding tongue, low set dysplastic ears, cochlear deafness, short neck, brachydactyly and abnormal creases, short broad 1<sup>st</sup> toe and generalized hirsutism. Brain MRI showed an empty sella. X-rays showed wormian bones, no sclerosis of the skull, short hand phalanges. His karyotype was 46,XY. At that time Schinzel-Giedion syndrome was diagnosed. In 2008 Affy 250K Nspl arrays showed a submicroscopic der(9)t(9q34;17q25). This represents one of the largest 9q34 microdeletions described, probably explaining the severe phenotype. Hirsutism, hydronephrosis and severe neurological phenotype are typical of SGS and unusual for 9q34 deletions. Our observation, however, arises the question whether at least some SGS patients might have a cryptic chromosomal imbalance or, less likely, whether some of the genes on 9q34 in our patient contain a second recessive mutation.

#### P02.104

##### Novel Findings By Genome-wide Copy Number Analysis on Chromosome 22 in a Case with Mild Facial Dysmorphology and Autistic/Schizophrenic Behaviours

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Autism and schizophrenia are two neuro-psychological disorders which may occur superimposed on each other. They can be accompanied with some dysmorphic syndromes such as Fragile X syndrome, Williams syndrome and Down syndrome. We present a 13-year-old female case who showed autistic symptoms at first and followed by positive schizophrenic symptoms. She also had multiple mild dysmorphic features such as elfin-like facial features, arched eyebrows, hypertelorism, depressed nasal bridge, bulbous nasal tip, downturned corners of mouth, thick lower lip and joint laxity. Karyotyping and FISH for Williams, Angelman and subtelomere of 22q were performed. We performed whole genome copy number analysis by Affymetrix Gene Chip 6.0 array for both case and parents. We showed inherited copy number variations without any additional region. We also performed LOH analysis and showed ~ 7Mb homozygosity at 22q11.

No dysmorphic syndrome was identifiable; therefore she could be an example of an unusual clinical picture with positive autistic, psychotic

symptoms, speech, language disability and multiple minor anomalies.

#### P02.105

#### Spondyloepiphyseal dysplasia tarda with progressive arthropathy (SEDT-PA) in siblings

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Spondyloepiphyseal dysplasia tarda with progressive arthropathy (SEDT-PA OMIM#208230) also known as Progressive Pseudorheumatoid Arthropathy of Childhood (PPAC) is a very rare autosomal recessive skeletal dysplasia with an estimated incidence of 1 per million in the UK. It is characterised by a postnatal progressive chondropathy affecting primarily articular cartilage. Clinically, the condition mimics Juvenile Rheumatoid Arthritis, and the diagnosis is often significantly delayed. We want to raise awareness of this debilitating condition and present 2 siblings from a non-consanguineous family with SEDT-PA diagnosed radiologically. They presented with severe progressive pain and stiffness of the hip joints, waddling gait and rheumatoid-like hands from 3 years of age. Their X-rays showed generalised osteopenia, platyspondyly, narrow joint spaces, metaphyseal widening and flattening of epiphyses. SEDT-PA was confirmed by identifying compound heterozygote mutations in the Wnt1-inducible secreted protein 3 (WISP3) gene. The WISP3 protein is a member of the CCN family (Cysteine-rich 61, Connective tissue growth factor, Nephroblastoma overexpressed) of secreted proteins that specifically associate with extracellular matrix. CCNs are primarily maintenance proteins that modify cellular responses to environmental factors and stimuli. Absence of WISP3 interferes with normal regulation of postnatal skeletal growth and cartilage homeostasis leading to precocious joint degeneration. The older sibling is on a trial treatment with Bisphosphonates in view of her marked osteopenia.

#### P02.106

#### Gender affects clinical suspicion of Down syndrome

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Recent study suggested that low male to female ratio (sex ratio, SR) in patients with only clinical diagnosis of Down syndrome (DS) was due to a sex bias in clinical diagnosis (Kovaleva NV, AJHG 69, S4:296). OBJECTIVES: (1) to determine a proportion of misdiagnosed cases among children tested for suspicion of trisomy 21, and (2) to study SR among those not having trisomy 21 according to their age at the genetic investigation. STUDY POPULATION: children referred to cytogenetic testing because of having clinical features resembling DS, born in 1970-2008. RESULTS: Among 1197 children born in 1986-2008, when completeness of cytogenetic confirmation of trisomy 21 had been improving from 86% to about 100%, there were 96 (8%) with normal karyotype (annual rate varied from 0% in 1990 to 19% in 2008). Overall, normal karyotype was diagnosed in 99 newborns (16M/83F, SR=0.19, p<0.0001), in 68 babies of the age up to 1 year old (25M/43F, SR=0.58, p=0.01), and in 59 children aged 1 year and older (27M/32F, SR=0.84, p>0.05). Thus there was a strong female prevalence in misdiagnosed newborns decreasing to about population value of 1.06 when children growing up. CONCLUSION: the data obtained suggest that gender affects clinical suspicion of DS. Since characteristic features allowing suspicion of DS include facial dysmorphism, one may hypothesize sex differences in the normal process of facial cranium ontogenesis during perinatal period. An abnormal condition(s) specific to females might also be implicated in a proportion of the misdiagnosed cases. Data of the follow-up study will be presented.

#### P02.107

#### A girl with short stature due to SHOX deletion inherited from paternal Y

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Short stature is a frequent disorder for which clinical attention is required during childhood. Short stature homeobox-containing gene

(SHOX) is located on the pseudoautosomal region (PAR1) of the short arm of the X and Y chromosomes. SHOX gene haploinsufficiency, due to microdeletions or intragenic mutations, causes a highly variable phenotype, ranging from isolated short stature to Leri-Weill dyschondrosteosis (LWD), while nullizygosity results in Langer mesomelic dysplasia (LMD).

We described the clinical and cytogenetic findings of a familial case of SHOX haploinsufficiency due to a microdeletion inherited by an unusual mechanism. The proband, a six-year old girl, was referred to our clinic for growth failure. On physical examination she presented disproportionate short stature with mesomelic shortening of the limbs and appearance of muscular hypertrophy. There were no dysmorphic features, except relative macrocephaly and high arched palate. X-rays of the forearms showed no evidence of Madelung deformity. Endocrine studies and karyotype were normal. Fluorescence *in situ* hybridization (FISH), using a SHOX probe, revealed a microdeletion on one of the X chromosomes in the girl - 46,XX.ish del(X)(p22.3p22.3)(SHOX-)pat -, and on the Y chromosome in her father - 46,XY.ish del(Y)(p11.3p11.3)(SHOX-) -, who also shared similar features. These results suggest a prior meiotic recombination event on the father to account for the transfer of the deleted SHOX gene to the alternate sex chromosome. Only three cases of inherited SHOX deletions by the same mechanism have been reported. The recognition of the etiology of short stature in this family allowed an adequate genetic counseling.

#### P02.108

#### Silver-Russell syndrome: a case report

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We report on a 33 years old male, with moderate adult Silver-Russell syndrome presentation. He was born at 41 weeks of gestation by spontaneous delivery with intrauterine growth retardation. Birth length was 46cm (-2 SDS), birth weight was 2200g (-3.5 SDS), and occipitofrontal circumference was 35cm (+1SDS). At 1 month he was suspected to develop hydrocephalus and at 1 year, because of prominent skin scalp vessels and systolic murmur, he was thought to present cerebral vascular malformation. Additional investigations were all normal. At 12 years, ultrasonography and skeletal x-rays showed asymmetric kidneys and delayed bone age. He grew up without feeding difficulties (BMI= -2 SDS at 2 years old) and did not receive growth hormone therapy; he demonstrated excessive sweating during childhood. Now, at 33 years old, his final height is 152cm (-3.5 SDS) and he presents a slight lower limbs asymmetry, relative macrocephaly (+1.5 SDS), bilateral fifth finger clinodactyly, and triangular face.

Methylation analysis of the 11p15 ICR1 region was performed and indicated loss of methylation, leading to biallelic expression of *H19* and loss of expression of *IGF2*.

We would like to emphasize the relative good clinical evolution of this male patient without hormonotherapy, nor feeding enteral requirement even though his short stature is a real socio-professional disability which is not enough recognized by the institutions. We speculate that given the difficulty of assessing this diagnosis especially in adults, this syndrome must be underdiagnosed until molecular analysis is performed.

#### P02.109

#### A family with Spinocerebellar ataxia type 8, Friedreich's ataxia and Hemoglobin S

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Spinocerebellar ataxias (SCA) are a heredodegenerative disease. It is classified according to the clinical signs, affected neuroanatomical regions and genetic features. SCA type 8 is characterized by gait and limb ataxia, dysarthria, nystagmus, pyramidal findings and decreased

deep sensation. SCA 8 is caused by an expanded (CTG) trinucleotide repeat on the chromosome 13. Friedreich's ataxia (FRDA), the most common subtype of early onset hereditary (SCA), is an autosomal recessive neurodegenerative disorder caused by unstable GAA tri-nucleotide expansions in the first intron of FRDA gene located at 9q13-q21.1 position. Clinical findings are characterized by neurologic signals and symptoms of the dorsal root ganglia, the posterior columns, and pyramidal and spinocerebellar tracts. Here we reported a family who was affected by SCA 8, FRDA and Hemoglobin S. Gait ataxia was the first symptom in index cases, followed by dysarthria, weakness in lower distal limbs and decreased deep sensation and deep tendon reflexes respectively. Cerebral MRI showed pure cerebellar atrophy in patient. An spinocerebellar ataxia and HbS were diagnosis on the virtue of family history, neurological examination and laboratory and genetics studies. Genetic studies disclosed a mutation on the SCA 8 locus and FRDA. Index case was homozygous for FRDA (675/775), his mother (normal/775) and father were heterozygous, (normal/675). In addition we determined HbS trait in some person in family.

Spinocerebellar ataxias are a group of disorders classified according to associating clinical signs and symptoms. To the best of our knowledge, this unusual finding has not been reported previously in the literature.

#### P02.110

##### Tongue anomalies in clinical genetics evaluation - Iasi medical genetics center's experience

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Traditionally the oral area receives minimal emphasis in the medical examination. Tongue birth defects are among the most common anomalies and require a careful clinical evaluation. The purpose of this study is to facilitate the clinical diagnosis of a syndrome with tongue anomaly.

We selected 16 patients (8 boys and 8 girls) from the total patients evaluated in Iasi medical genetics centre during 5 years (2000 - 2004, 8615 patients). We studied all tongue anomalies according dysmorphic terms defined by Carey JC et al, 2009. We selected those anomalies with a high power to suggest the diagnosis (evocative anomalies): macroglossia, lobulated tongue, microglossia/ hypoglossia; bifid tongue, asymmetric tongue, tongue fasciculation. We observe the preponderance of following traits: macroglossia (10 cases - 62.5%), Microglossia (2), Lobulated tongue (1), bifid tongue (1), asymmetric tongue (1), tongue fasciculation (1). The syndromes diagnosed were: congenital hypothyroidism (7 cases), Down syndrome (1), hypoglossia-hypodactylia spectrum (3), MEN IIB (1), Opitz G/BBB (1), Werdnig Hoffmann (1) and multiple anomalies (2). The tongue anomalies were diagnosed after 2 months age old because of the tongue role in mastication, deglutition, speech etc. The small number of cases could be explained by the low frequency of the anomalies or by missing the diagnosis owing to a facile clinical evaluation.

For accurate assessment, correct diagnosis, and management, the patients should be dealt with in a team approach. When the genetic tests are budget limited a clinical proper diagnosis is essential to initiate the correct treatment and genetic counseling.

#### P02.111

##### Tracheoesophageal fistula is not significantly associated with intestinal malrotation - a study based on the Glasgow Register of Congenital Anomalies and NorCAS database

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*Western Infirmary (NHS Greater Glasgow & Clyde), Glasgow, United Kingdom.* Numerous and large epidemiological studies have been undertaken of tracheoesophageal fistula (TOF). Only a single study has been published specifically looking at the epidemiology of intestinal malrotation (Forrester and Merz, 2003). The Glasgow Register of Congenital Anomalies was used to study anomalies associated with cases of intestinal malrotation in babies born between 1997 and 2005 (30 cases, 15 syndromic) in Glasgow, UK, and findings were compared with a previous study (157 cases, 70 syndromic) (Munir S, Muneer A, Intestinal malrotation and Hedgehog signaling defects - an epidemiologic study based on the NorCAS database and London Dysmorphology Database. [Abstract 631]. Presented at the annual meeting of the

American Society of Human Genetics, Oct 26, 2007).

Only 2 cases of TOF (2.8%) were seen in the NorCAS study and none in the current study or in Forrester and Merz's study. Moreover, while 17 cases (27%) of syndromic malrotation in the NorCAS study had features of VACTERL, and 2 cases (13%) in the current study, and 2 cases in Forrester and Merz's study, none of them exhibited TOF. Mutant mouse models with Hedgehog signaling defects exhibit intestinal malrotation and features of VACTERL, including TOF. Whilst it has yet to be seen whether and what proportion of humans with malrotation and VACTERL have Hedgehog signaling defects, the striking absence of TOF from VACTERL in cases of syndromic intestinal malrotation as seen in these studies, and virtual absence of TOF in known hedgehogopathies such as Pallister-Hall Syndrome, merits further investigation for differences between hedgehog signaling in mice and humans.

#### P02.112

##### Familial congenital unilateral cerebral ventriculomegaly: Delineation of a distinct genetic disorder

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We identified 2 female siblings, derived from healthy first cousin parents, with congenital unilateral cerebral ventriculomegaly detected prenatally. Patient 1 underwent ventriculoperitoneal shunt operation at 1 week old, while patient 2 was followed without surgical intervention. Both patients presented with mild developmental delay and hemiparesis contralateral to the involved hemisphere. Focal seizures were observed in patient 1, whose neuroimaging revealed posterior insular polymicrogyria in the normal sized ventricle hemisphere and retrocerbellar cyst. Both siblings displayed near absence of white matter with marked thinning of the overlying cortex in the affected hemisphere and very thin corpus callosum. Investigations revealed no other system involvement and karyotyping was normal. Normal TORCH screening in subsequent pregnancies, normal parental coagulation profile and undetectable maternal autoantibodies suggested against the possible role of extrinsic factors as an etiological factor for unilateral ventriculomegaly. Parents had normal brain imaging findings. To our knowledge, unilateral ventriculomegaly has never been reported with familial clustering. We suggest delineation of a distinct developmental brain defect, most likely of autosomal recessive inheritance.

#### P02.113

##### Clinical genetic analysis of non system vasculitis of small vessels in children.

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Currently there are no any known specific markers for differential diagnostics of the majority of vasculitides.

Objective: to identify the most informative traits, with the aim to facilitate classification in two groups vasculitides of small vessels in children.

Patients And Methods: 80 children with vasculitides of small vessels aged from 3 to 17 years. Among them, 46 patients suffered from system vasculitis (purples of Shenieja-Genoh, PSG) and 34 suffered from non system vasculitis (purples of Schamberg, PS). Control group included 144 healthy individuals. Diagnoses were verified by clinical, laboratory and morphological methods (skin biopsy). 4a/4b, T-786C, G894T polymorphisms of the gene of endothelial NO synthase (eNOS) were studied by standard methods.

Results: Two distinct forms of PS were identified: sharp cyclic clinical course in 12 patients (36 %) and chronic recurrent course in 22 patients (64 %).

Hemosiderin in skin biopsy was found in 5 patients with the sharp course of the disease and in 6 patients with the chronic form of PS. aa genotype of 4a/4b polymorphism was found more often in patients with

both vasculites compared to the control ( $p = 0.001$ ). G allele of G894T polymorphism was found to be more frequent patients with vasculites compared to the control ( $p = 0.001$ ).

Conclusion: 4a/4b and G894T polymorphisms were appeared to be the most informative traits helping differential diagnostics of both groups of the vasculites (purples Shoenlein-Genaha and purples Schamberg).

#### P02.114

##### Antithrombin Ala383Pro: a new missense variant identified in a patient with venous thrombosis

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Our aim was to study a new mutation in the *SERPINC1* gene in a patient with venous thrombosis. We performed analysis of Antithrombin (AT) Cambridge II (*SERPINC1* G13268T; p.Ala384Ser) using PCR followed by digestion and it was confirmed by direct sequencing to distinguish it from the AT Cambridge I (G13268C; p.Ala384Pro). We determined functional AT (AT-f) and antigen AT (AT-Ag) and the mobility of AT by crossed-immunolectrophoresis (CIE) in the presence of heparin. Also Protein C, Protein S, activated protein C resistance, antiphospholipids antibodies, lupus anticoagulant, Factor V Leiden, F2G20210A, F12C46T were determined. The patient had a qualitative (type II) AT deficiency (AT-f:54%, AT-Ag: 95% with a normal heparin binding pattern in CIE). The results suggested that proband was a carrier of AT Cambridge. However, sequencing analysis revealed a new genetic variant within exon 6 of the *SERPINC1* gene, G13265C. This mutation result in the exchange of residue Ala383 by a proline, 10 residues N-terminally of the Arg393-Ser394 scissile peptide bond located in the reactive center loop and it was not detected in 100 healthy subjects. It is likely that this phenotype is a substrate-like behaviour of mutant AT molecules, as the bulkier proline residue would not be able to insert rapidly into the main body of the serpin after thrombin/FXa-mediated cleavage of the Arg393-Ser394 peptide bond. Moreover, the presence of this new variant could interfere with the diagnosis of mutation Antithrombin Cambridge II.

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#### P02.115

##### Waardenburg syndrome type II: about 4 cases

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Waardenburg syndrome (WS) is a hereditary auditory-pigmentary syndrome, the major symptoms being congenital sensorineural hearing loss and pigmentary disturbance of eyes, hair and skin. Depending in additional symptoms, WS can be classified into four types: WS type I (WS1) is associated with facial deformity such as dystopia canthorum; WS2 has no other symptoms; WS3 is associated with upper limb deformity; and WS4, with megacolon.

It is an autosomal dominant affection which has a wide heterogeneity. We describe here the clinical manifestations of 4 patients.

The first case was a familiar case in which the father and his son have congenital deafness, heterochromia irides, and a slight scoliosis.

The second case is an isolated case of a boy aged of 18 months, having severe deafness, hypochromic irides and ocular albinism.

In the last case we report an eight year-old boy who got a partial albinism (white forelock, hypopigmented skin lesions) associated with dysmorphic features and mental retardation. Audiometry was normal. Our patients had clinical features compatible with Waardenburg syndrome type II.

Type II Waardenburg are a heterogeneous group with normally located canthi, sensorineural, hearing loss (77%) and heterochromia iridum (47%).

Two genes are mutated in this form et should be screened.

#### P02.116

##### X-linked agammaglobulinemia: the underlying genetic defect in three families

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X-linked agammaglobulinemia (XLA) is a heritable immunodeficiency disorder that is caused by a differentiation arrest in the bone marrow resulting in severe B cell deficiency. It is caused by mutations in the Bruton's tyrosine kinase gene (Btk), that encodes a pathological tyrosine kinase with a pivotal role in the life cycle of B cells. To date, more than 550 mutations have been identified scattered along the entire length of the Btk gene. We investigated three unrelated patients with clinical diagnosis of XLA and their mothers for mutations in the Btk gene. Two patients were found to have chain-termination mutations in the kinase domain: a 4 bp deletion at positions 527-528 resulting in frame shift and a premature termination codon at position 528, and a nonsense mutation at codon 520 at the second patient. The third patient has a missense mutation c.29T<A in exon 2. All patient's mothers were proved to be heterozygous for the mutation found in her sons. Mutation detection in the Btk gene provides a definitive diagnosis in X-linked agammaglobulinemia, indispensable for adequate genetic counseling and carrier detection.

#### P02.117

##### Detection of an heterozygous R553G Cystic Fibrosis Mutation in routine carrier screening in Greece

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Cystic Fibrosis (CF) is the most common autosomal recessive disease in Caucasians. The carrier frequency is approximately 5 % in North Europe, while among the Greek population, cystic fibrosis constitutes the second most common disease after β-thalassemia. A 35-year-old Caucasian male chose to have mutation screening although there was no family history of CF. Cystic fibrosis transmembrane conductance regulator (CFTR) mutation analysis using the Inno-LiPa CFTR assay revealed presence of hybridization for both the wild-type and mutant oligonucleotides for R553X mutation. The Tag-IT CFTR 40+4 assay was also used for further testing of the sample. Upon analysis of the patient results no mutant allele was detected for the mutation R553X. The patient seemed to be normal in all mutations screened. This region was sequenced, and an apparent heterozygous for R553G mutation was detected. This is the second time this mutation is found but not in a patient but in a healthy carrier with no symptoms. As shown in this case the method used for carrier screening is also of high importance in order to determine the actual mutation. Technical anomalies leading to the incorrect interpretation of the patient's results cannot be excluded. This case demonstrates that counseling patients who meet laboratory criteria for a CF diagnosis but do not have clinical symptoms of CF is problematic. The rare mutations detected in these nonsymptomatic patients may not represent independent disease-causing mutations, and care is advised in offering phenotypic predictions.

#### P02.118

##### The French Cystic Fibrosis Laboratory Network: seven years' experience

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CFTR gene studies represent one of the most frequent genetic analyses performed worldwide. In order to ensure provision of best genetic services in this field, 35 molecular genetics laboratories organized the French CF Laboratory Network. This network, the implementation of which was facilitated by the European CF Network, was recognized in 2001 by the French Ministry of Health. Ten laboratories received financial support for in-depth CFTR gene studies and a subgroup of four had additional support, as reference centres, for management of the Network and clinical research.

Based on best practices guidelines for CFTR gene analysis, DNA samples may be processed in one or two different laboratories, depending on the indication of the test and the laboratories' level of expertise. More than 10,000 samples are studied per year (50-1500 per lab) in

cluding 200 for prenatal diagnosis. The continuous share of experience with rare variants greatly helps genetic counselling. Since 2001, six workshops were organized, convening molecular geneticists, technicians, clinicians of CF care centres and private companies. Three main topics were addressed: 1) Network functioning, discussion on best practice guidelines and report on European actions; 2) technical aspects including new technologies; 3) and clinical issues. A number of collaborative studies have been published (e.g. Claustrès, Hum Mutat 2000; Muller, Am J Med Genet 2002; Feldmann, Hum Mutat 2003) and others are still ongoing, such as the study of phenotype-genotype correlations on R117H. The involvement of several of the Network members in European actions contributes to the dynamism of the French Network.

#### P02.119

##### The prevalence of common CFTR gene mutations and 5T allele between infertile men in Russia

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Introduction: Cystic fibrosis conductance transmembrane regulator (CFTR) gene mutations are common genetic cause of male infertility. The frequency and the spectrum of CFTR mutations are variable between populations.

Materials and methods: We examined a cohort of 850 men from Russian infertile couples. Fourteen common CFTR mutations (CFTRdele2,3(21kb), 394delTT, L138ins, R334W, F508del, I507del, 1677delTA, G542X, 2143delT, 2184insA, 3821delT, W1282X, N1303K and 3849+10kbC>T) and Tn-polymorphism in the intron 8 (IVS8T) have been analyzed.

Results: CFTR gene mutations were found in 40 (4.7%) patients. Following heterozygous mutations have been revealed: F508del (n=17), CFTRdele2,3 (n=7), W1282X (n=6), 2143delT (n=4), 2184insA (n=1), G542X (n=1), R334W (n=1), 1677delTA (n=1), 3849+10kbC>T (n=1) and 3821delT (n=1). The presence of 5T allele was found in 96 (11.3%) infertile men. This CFTR allele has been revealed in the heterozygous (87.5%), in the homozygous (4.2%) and in the compound heterozygote state (8.3%) with following common CFTR mutation: F508del (n=4), CFTRdele2,3 (n=1), W1282X (n=2) and G542X (n=1). Mild (atypical) forms of cystic fibrosis (CF) with extragenital features of CF (with a presence of chronic bronchitis or chronic pancreatitis) were diagnosed in 4 (0.5%) infertile men. CBAVD and/or obstructive azoospermia were revealed in about 2% of examined individuals. One severe oligozoospermic patient presented with unilateral renal agenesis (URA) associated with CUAVD syndrome.

Conclusion: Obtained results demonstrated high prevalence of common CFTR mutations and 5T allele between Russian infertile men. Following four mutations: F508del, CFTRdele2,3, W1282X and 2143delT, are the commonest CFTR mutations in Russia.

#### P02.120

##### Prenatal diagnosis by ARMS-PCR and genetic counseling in couples of CFTR mutations carriers

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Objectives: The aim of this study was to provide a correct prenatal diagnosis and genetic counseling for couples with previous family history of cystic fibrosis who intended to have a new child.

Method and materials: 11 couples were selected for prenatal diagnosis, based on family history or typical echographic findings. The couples had previous children with cystic fibrosis who had been genetically tested and had both mutations identified or had deceased children diagnosed with cystic fibrosis but without molecular diagnostic. Genomic DNA was isolated from amniotic fluid collected by transabdominal amniocentesis in the 16th week of pregnancy and from venous blood collected on EDTA from both parents. The genetic analysis for CFTR mutations was performed with Elucigene CF29 kit which uses the ARMS-PCR method. STR genotyping was performed for several

loci in order to verify the absence of contamination with maternal blood or cells. When maternal contamination of amniotic fluid was unavoidable, the culture of amniocytes in specific media was used for selection of fetal cells.

Results: 4 heterozygous genotypes were identified (ΔF508/N, G542X/N), six normal genotypes and one compound heterozygous genotype (621+1G>T/ΔF508).

Conclusions: It is possible to perform prenatal diagnosis using Elucigene CF29 kit in all cases with the exception of cases which can be homozygous for non-ΔF508 mutations. STR genotyping and amniocytes culture are complementary methods used in difficult cases for obtaining a correct prenatal diagnosis.

#### P02.121

##### Prenatal diagnosis in CF Russian families

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Cystic fibrosis is the most common recessive disease in Caucasians. The spectrum and relative frequencies of mutations in the CFTR gene significantly varied between different populations and ethnic groups. According to our previous investigations 20 different CF mutations (CFTRdele2,3(21kb), 394delTT, 604insA, L138ins, 621+1G>T, R334W, R347P, F508del, 1677delTA, G542X, 2143delT, 2184insA, K598ins, 3821delT, S1196X, 3677insTCAA, 3849+10kbC>T, W1282X, 3944delTG, N1303K) were accounted for 77% of CF alleles in Russian patients with F508del (54.2%) and CFTRdele2,3(21kb) (7.2%) being the most frequent ones. We have performed 123 prenatal diagnoses for 98 couples. Two cases included twin pregnancies: one homozygous and one heterozygous. 74 families with 17 different genotypes were fully informative for direct DNA analysis performed by using multiplexPCR, heteroduplex analysis and restriction analysis. In 19 families only one parent mutant allele was known and in 5 families none of CF mutations were identified, so prenatal analysis was done by indirect DNA analysis using haplotype analysis of four dinucleotide repeats (IVS1CA, IVS8CA, IVS17bCA, W30), one tetranucleotide repeat (IVS6aGATT) and four biallelic restriction polymorphic sites. Materials used for fetal DNA analysis were CVS samples. 30 fetuses were affected, 66 - heterozygous carriers and 28 were healthy with two normal alleles of CFTR gene. In all cases when prenatal diagnosis indicated that the fetus had CF the couples chose pregnancy termination.

#### P02.122

##### A new cryptic CFTR exon in mild Cystic Fibrosis

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Cystic fibrosis (CF) is the most frequent autosomal recessive disease in the Caucasian population. So far, over 1500 CFTR gene mutations have been described responsible for classical CF and CFTR-related disorders. Screening for large gene rearrangements has improved identification of CF alleles but a number of cases remain unsolved, making genetic counselling difficult, particularly when the diagnosis is uncertain. Unidentified CF mutations may lie in introns, such as 3849+10kbC>T and 1811+1.6kbA>G.

We report the case of a French 25y woman diagnosed with disseminated bronchiectasis, pancreatic sufficiency and borderline sweat tests. Screening of the 27 exons including search for gene rearrangements only identified F508del in heterozygosity. Investigation at the mRNA level was then performed from nasal epithelial cells.

An abnormal, longer CFTR mRNA was detected, corresponding to a 97bp insert between exons 6b and 7. This fragment matches a 101bp sequence of intron 6b, and is flanked by the consensus donor and acceptor splice sites. A new stop codon is created within the inserted sequence. The amount of abnormal CFTR mRNA of the patient was estimated to be 20% of the wild type, which suggests the abnormal splicing is due to partial activation of a cryptic splice site. This hypothesis is supported by the fact that the patient does not suffer from severe CF. Studies at the genomic level are still ongoing to identify the mutation which leads to partial activation of a cryptic splice site and explains the creation of a new exon.

## P02.123

**A synonymous mutation in the CFTR gene causes an aberrant splicing in an Italian patient affected by cystic fibrosis.**

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Genotype screening in human disease frequently identifies exon/ intron sequence variations whose association with the disease phenotype is unclear. In fact the pathologic effect of an apparently benign polymorphism, such as codon third position variations, or nucleotide change/ deletion in the intronic non canonical splicing regulatory elements, are difficult to assess.

In this work we define the pathogenic role of a synonymous mutation found in an Italian patient affected by cystic fibrosis (CF). It consists of a G>T substitution at nucleotide 2811 on exon 15 of CFTR gene. CFTR mRNA analysis showed that this synonymous mutation created a new 5' splice site inside exon 15 causing the skipping of 76 amino acids.

Furthermore, CFTR mRNA analysis showed that, although this aberrant splicing caused a shorter exon 15, the downstream exonic sequence from exon 16 to the end of the ORF was in frame.

Therefore, the skipping of amino acid residues included in exon 15, located in CFTR transmembrane domain MSD7, probably modify the structure and the regulation of the Cl<sup>-</sup> channel.

The growing interest on the potential effects of single-nucleotide changes in coding and non-coding regions on the extent and accuracy of pre-mRNA splicing is expected to have a significant impact on the diagnosis and treatment of genetic diseases. The correct classification of mutations is essential to understand structure-function relationship in the corresponding protein, to assess the phenotypic risk in CF patients and to devise new therapies.

## P02.124

**Identification of a novel CFTR mutation in a patient with cystic fibrosis**

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Cystic fibrosis (CF) is a most common autosomal recessive disease with a prevalence of 1 in 2500 and a carrier frequency of 1:22. CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene expressed in several organs including the lung, the pancreas, and the sweat glands. So far 1604 mutations of the CFTR gene have been reported at the CF Mutation Database. We examined a 17-year-old girl, a daughter of a Hungarian mother and a Nigerian father with a tentative diagnosis of CF. The patient's history was uneventful until her school years when mild upper respiratory infections appeared with repeated coughing periods and sweat-chloride levels of 102mmol/l, however she did not have any intestinal symptoms. As direct sequencing of all 27 exons of CFTR revealed, the patient had two mutations in compound heterozygous form: a 2183 AA>G frameshift mutation in exon 13, described previously in three unrelated CF patients of Sardinian descent; and a mutation not yet reported elsewhere, a 3872 G>A transition in exon 20 resulting in a glycine-glutamate amino acid change at position 1247. We found that the 2183 AA>G frameshift mutation was inherited from her Hungarian mother, while the 3872 G>A transition from her Nigerian father. The latter is also carried by her brother. This novel mutation affects an ATP-binding site of the CFTR protein, and was predicted to be probably damaging by the Poly-Phen prediction software, thus we presume that it is a CF-mutation causing a milder, late-onset form of CF.

## P02.125

**Distribution of CFTR mutations in cystic fibrosis patients from Eastern Hungary**

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Cystic fibrosis (CF) is the most common severe inherited monogenic disease in Caucasians. In addition of the most frequent deltaF508 mutation which is responsible for the 50-65% of the cases, more than 1300 different rare mutations are present in the CFTR gene. Distribution of the different CFTR mutations show large ethnic variability. As an initial attempt to create a mutation testing panel in Hungary, the aim of this study was to determine which mutations are present among Eastern Hungarian CF patients.

For the mutation analysis, 43 patients with severe CF phenotype were selected and tested with commercially available multiplex allele specific PCR and oligonucleotide ligation methods.

Mutations in both alleles could be identified in 28 patients (65%). 19 patients (44%) were homozygous for deltaF508, while the other 9 patients were compound heterozygous for two mutations. 4 out of them had deltaF508 and CFTRdele2,3(21kb), 2 had deltaF508 and N1303K and one patient had deltaF508 with c.1717-1G>A. The remaining two patients possessed two rare mutations (G542X with N1303K and G542X with R347P).

In 12 patients (28%), only one mutation could be detected. 10 patients were heterozygous for delta F508, one patient had G542X and an other had N1303K in heterozygous form. In 3 patients (7%) no mutation was found.

In conclusion, using multiplex mutation detection methods, the complete underlying genetic background could be identified in 65% of CF patients from Eastern Hungary, while in other 28%, one mutation was found. The frequency of CFTRdele2,3(21kb) mutation with Slavic origin was surprisingly high (9%).

## P02.126

**Cystic fibrosis liver disease and CFTR or MBL**

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Background: CFTR (cystic fibrosis transmembrane conductance regulator) gene and MBL (mannose binding lectine) gene seems to influence development of CF liver disease. Severe CFTR genotype and low serum MBL are promoting factor in development of CFLD. Study aim: assessment of CFLD patients in relation with genotype and MBL levels. Methods: Twenty five children with CFLD, followed in our National CF Centre were evaluated. Genotype results were taken from centre's data base. MBL assay procedure was performed using MBL oligomer ELISA kit. Results: In patients with CFLD, 36% were MBL deficient; from those 66% Δ F508 homozygous, while in patients with normal or increase MBL 56% patients had "severe" genotype. Δ F508 homozygous genotype was found in 43% CFLD patients, each other with a different genotype. Conclusions: A correlation between CFLD and specific genotype or MBL level could not be made. MBL2 genotyping is necessary to identify the patients predisposed to develop CFLD. Heterogeneity of genotypes in CFLD patients suggests the hypothesis of other factors in determination of cystic fibrosis associated liver disease.

## P02.127

**Abnormal ultrasound signs of fetal bowel in cystic fibrosis: a molecular approach**

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Hyperechogenic fetal bowel (HFB), a possible prenatal sign of CF, is detected in 0.1-1.8% of pregnancies during the second or third trimester. The prevalence of meconium ileus (MI) is higher among neonates with previous HFB than in neonates with normal ultrasound imaging so there could be a relationship between HFB and MI.

The aim was to evaluate usefulness and limits of genetic analysis in

couples with HFB, defining the best testing strategy based on a review of CF patients with MI.

Results of genetic analysis on 79 Italian CF patients with MI, and 47 HFB couples were reviewed.

Screening for the most frequent mutations in 79 MI patients allowed a 90.5% detection rate, which increased to 96.8% after DNA sequencing, and to 98.1% after MLPA; in two couples of carriers was confirmed CF in the fetus. A third couple terminated the pregnancy of a child F508del/P5L. In a further pregnancy the fetus F508del heterozygote again had HFB. Because of the severity of the ultrasound picture, this pregnancy was terminated. Testing the most frequent mutations and deletions covers 91.8% of CFTR mutations in CF with MI. A similar testing strategy may be appropriate in HFB. We do not suggest to sequence the gene: it's possible to identify mutations with unknown/unclear clinical consequences. We also suggest to proceed with extreme caution when counselling parents of a fetus with HFB who are carriers of mild or novel mutations, as their presence in the fetus does not justify a diagnosis of CF.

#### P02.128

##### Polymorphism of cystic fibrosis in Moldavian patients

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The aim of presented study was to determine clinical features in patients with cystic fibrosis (CF) in correlation with types of mutations in CFTR-gene.

Were investigated 123 patients with CF aged from 2 months to 24 years (55 female and 68 male), during the period 1992 - 2008. Were determined 9 mutations (F508del, G542X, N1303K, W1282X, R117H, G551S, R347P, R334W, R553X). In 3,2% of patients the diagnosis carried out in France and Russia. Adult patients were 18,7%. In 91,9% of cases was mixed CF, in 4,9% - pulmonary and in 3,2% - intestinal CF. The course of CF was severe in 59,4% of cases, moderate in 40,6%. We detected F508del mutation in 57,7% of patients, 16,26% homozygous, 41,46% heterozygous, suffered from severe mixed CF, 39,1% of them died. Were revealed heterozygous mutations: R334W in 2,4%, N1303K in 0,8%, G542X in 0,8% of cases, and 3849+10kbC>T in 0,8% of cases, compounds in 2,4% (F508del/185+1G>T; G542X/ N1303K; 128+1G>A/1677 del); the type of CF was mixed, and course was severe with early manifestations. Mutations were identified in 65,8% of cases, in 43,9% one, in 18,7% two mutations and in 34,2% mutations were not identified.

Conclusions. The prognosis and severity of CF in Moldova was determined not only by F508del mutations which frequency was 57,7%, but some mutations in compound, which suggest the necessity to enlarge spectrum of detecting mutations. It is possible the influence of non-identified major mutation in Moldavian population.

#### P02.129

##### Assessment of a common variant repeat sequence within a cystic fibrosis newborn screening program

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We have assessed the heterozygote frequency among neonates with hypertrypsinemia (elevated IRT) detected through a provincial CF newborn screening program by monitoring the CFTR mutation spectrum and frequency, including the IVS8-5T variant and tandem TG-tract lengths. The Luminex xTAG™ Cystic Fibrosis Kit, which detects 39 cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations as well as 4 CFTR variants was used for molecular analysis. A total of 116,550 neonates were screened for CF using a top 2% cutoff for mutation analysis. Of the 2,331 newborns with increased IRT, one mutation was detected in 176 (heterozygote frequency of 7.6%), or about 2 times greater than in the general population. IVS8 T-tract alleles were only reported in newborns with R117H mutations, in accordance with ACMG recommendations. However, all samples from IVS8-5T carriers were further genotyped for TG-tract lengths. In total, 220 5T alleles were detected (9.4% vs 10% in the general population). However, the 11TG allele had a frequency of 61% in 5T newborns with elevated IRT, as compared to 78% in the general population. Conversely, longer TG tracts were detected in the remaining

39% of newborns compared to 22% in the general population. We did not find an increased frequency of the 5T allele in neonates with hypertrypsinemia, however longer adjacent TG tracts, which are directly proportional to disease penetrance, are more frequent in this cohort of newborns. These findings may warrant further investigation into the applicability of 5T-TG genotyping within the panel of mutations used for CF newborn screening.

#### P02.130

##### Cystic Fibrosis in Rostov region

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Cystic Fibrosis is one kind of single-gene hereditary diseases caused by a mutation in the gene, cystic fibrosis transmembrane regulator. Cystic Fibrosis has high morbidity and mortality.

There is a trend of Cystic Fibrosis transforming lethal childhood disease into chronic illness of adolescents and adults. Prevalence of Cystic Fibrosis in Russian Federation is significantly lower comparing to North America and Europe. By now more than 1,000 gene mutations resulting in Cystic Fibrosis are known.

The most common gene mutations identified in the Russian Federation population are ΔF508 (53,0%), CFTRdel 2,3 (21kb) (6,4%), N1303K (2,6%), 2184insA (1,8%), 2143delT (1,8%).

Newborn screening for Cystic Fibrosis was introduced in 2006 in Russian Federation. This test measures raised blood concentrations of immunoreactive trypsinogen. Since newborn screening was implemented, 105263 children were tested and 139 children had elevated levels of immunoreactive trypsinogen. All children were followed up clinically. DNA analysis and sweat chloride tests were performed, too. DNA tests were done in order to identify 8 major mutations. According to the results of the test 80% of patients had ΔF508 heterozygote mutations. The prevalence of Cystic Fibrosis was 1:9500 in Rostov Region, which is similar with Russian Federation numbers. The most common genetic mutation was ΔF508. The introduction of newborn screening allowed to identify groups of children at risk. .

Modern DNA analyses allow us to establish diagnoses of Cystic Fibrosis and to implement appropriate therapy before clinical symptoms of the disease become apparent.

#### P02.131

##### New pathogenic mutation in cystic fibrosis patient

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Cystic fibrosis (CF) is an inherited disease that affects the respiratory tract, intestine and many body organs because of this called multisystem disease. In addition to affection of respiratory and digestive system, CF can lead to diabetes, polyps in nose and infertility in men. CF patients have shorter life expectancy. Cystic fibrosis (CF) is the most common severe autosomal recessive disorder in Caucasian populations and affects 1 in 2500 live births. Mutations in the gene Cystic Fibrosis Transmembrane conductance Regulatory (CFTR) which encodes a protein expressed in the apical membrane of exocrine epithelial cells, leads to this disorder. This gene has 27 exons. To date over 1400 different mutations in this gene have been identified.

The proband was a 3.5 years old that was born in a third degree familial marriage with a weight 3100 grams. Poor sucking, cough and diarrhea were seen from the first days of birth. At present, the chief complain of the patient are pulmonary sticky sputum and cough. The patient was analysed for mutations by PCR and sequencing methods for CFTR gene. The parents of patient were also analysed to confirm the found mutation.

A homozygous T deletion in exon 13, codon 754 was found in the patient DNA sample. This mutation in heterozygous state was found in parents. This mutation changes frameshift of Cystic Fibrosis Transmembrane conductance Regulatory protein.

We believed that this mutation is pathogenic. More investigation in cellular level needs to confirm our results.

#### P02.132

##### Mutation detection in sporadic NF1

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Sporadic neurofibromatosis type 1 (NF1) occurs in the absence of a family history of the disease and usually results from a new mutation in the germ cell of one of the parent. In most cases, the disease is caused either by mutation in the NF1 gene, or by a particular or complete deletion of the NF1 gene. The NF1 gene exhibits one of the highest mutation rates of any human disorder. Here we report preliminary data of the experimental NF1 gene study in patients from the Czech Republic. We have screening germinal and somatic mutations spectrum of 30 unrelated sporadic NF1 patients, using denaturing high-performance liquid chromatography (DHPLC) and multiplex ligation-dependent probe amplification (MLPA) assay. DNAs of all patients were isolated from peripheral blood and 10 cutaneous neurofibroma biopsies. By direct sequencing of the pre-selected amplicons we identified 6 causal germinal mutations, inclusive of 2 novel splice site mutations (c.2850+1 G >T, c.6641+1 G >A). All patients were examined by MLPA method and one of them harboured entire gene deletion.

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#### P02.133

#### Two PTPN11 gene mutations (Y63C and R501J) detected in two Tunisian Noonan syndrome's children

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Congenital heart defects (CHD) remain the most common birth defect, occurring in 1% of live births. Despite great advances in the CHD diagnosis and treatment, there continues to be significant associated mortality, morbidity and economic burden. The identification of genetic causes of CHD is important; to improve understanding of the aetiology of CHD and to promise the opportunity of a better prevention, diagnosis, and care.

In Noonan syndrome (NS) characterized by CHD and dysmorphic features, missense mutations of PTPN11 gene responsible of gain of function in the protein tyrosine phosphatase Shp2 account for approximately 50% of cases. At the Medical University of Sfax; Department of Histology; the pattern of PTPN11 mutations is defined in 15 NS Tunisian patients (and 3 mothers) using Bi-directional direct sequencing of PTPN11 exon 3 and its flanking intron boundaries. All patients harbour congenital pulmonary vein stenosis (PVS) (with or without other CHD) and NS facial dysmorphic features. Two mutations of the exon 3, Y63C (known mutation) and R501J (new mutation) are identified. Y63C, which is shown in a NS's patient with PVS associated to an ASD, affects the N-SH2 domain of SHP2. This mutation is not detected in the mother who has NS clinical phenotype. We suggest that the patient is a compound heterozygote with Y63C (de novo or paternal germline inherited) mutation and an other mutation inherited from the mother. R501J mutation that affect PTP domain of SHP2, was identified in a patient with NS features and an isolated PVS.

#### P02.134

#### Craniosynostosis in patients with Noonan syndrome caused by germline KRAS mutations

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Craniosynostosis, the premature fusion of one or more cranial sutures, is a developmental defect that disrupts the cranial morphogenetic program, leading to variable craniofacial dysmorphisms and associated functional abnormalities. Craniosynostosis is frequently observed as an associated feature in a number of clinically and genetically heterogeneous syndromic conditions, including a group of disorders caused by activating mutations of the fibroblast growth factor receptor family members FGFR1, FGFR2 and FGFR3. In these disorders, dysregulation of intracellular signaling promoted by the aberrant FGFR function is mediated, at least in part, by the RAS-MAPK transduction pathway. Mutations in RAS and other genes coding for proteins participating

in this signaling cascade have recently been identified as underlying Noonan syndrome and related disorders.

We have identified an identical germline KRAS mutation in two unrelated Noonan syndrome patients with a similar type of craniosynostosis. Although craniosynostosis does not seem to be an invariant feature of patients with this mutation, this finding highlights the significance of aberrant signaling mediated by the RAS pathway in the origin of craniosynostosis.

#### P02.135

#### Two cases of LEOPARD syndrome with same missense mutation in PTPN11 gene but different clinical manifestation

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LEOPARD syndrome (LS, OMIM 151100) a rare multiple anomalies condition belongs within of so called neuro-cardio-facial-cutaneous syndromes group. Mutations in PTPN11 and RAF1 gene are the only genes known to be associated with LS, mutation have been identified in about 93% affected individuals.

Patient 1 was born from first pregnancy with normal birth parameters. Parental main complaint was hyperactive behavior. First lentigines were presented at birth, but intensive growth started at the age of 2 years. Heart ultrasound showed mitral insufficiency. Patient 2 is second child in family born from the induced labor due to polyhydramnion. Hypertrophic cardiomyopathy (HCM) was diagnosed at the age of 1 month and closely followed by since to find out the etiological factor. She presented her first lentigines at birth, but rapid growth started only at the age of 3 years.

Patients were referred to the genetic consultation due to rapid growth of lentigines at the age of 4 years. They both had additionally slightly short stature, characteristic facial features. Molecular analyses was performed by bidirectional sequencing and revealed one of most frequently described PTPN11 gene missense mutation in LS, 836A→G (Tyr270Cys). Although patients have different health problems, rapid growth of lentigines in infancy lead to correct diagnosis. In literature mutation Tyr270Cys is more frequently associated with short stature, deafness and also with HCM. An earlier diagnosis of LS is useful for surveillance of the specific medical problems associated with LS and for precise genetic counseling to the family.

#### P02.136

#### A boy with atypical phenotype of NF1 with a type 1 microdeletion

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Patient was born as a first child of healthy, non-consanguineous Caucasian parents at week 38+5. During pregnancy hydronephrosis was seen at 19 weeks of gestation. Birth weight was 3890 g, length 47 cm and head circumference 35 cm. As a newborn dysmorphic facial features, anterior anus and an anocutaneal fistula was seen. The chromosomes (400 bands) were normal.

He walked independently at the age of 2 years 4 months, but did not speak. His height was 87.5 cm (slightly below his target height), body mass index (BMI) 16.2 and head circumference 53 cm. His facial features were distinct with high anterior hairline, thick eyebrows, epicanthal folds and hypertelorism. The nasal tip was broad and he had anteverted nostrils. The filtrum was long and the upper lip was thin. The ears were thickened and the ear lobules were uplifted. He had one classical cafe au lait spot.

In addition he has had severe feeding problems because of an intestinal malrotation. His MRI revealed high signal intensity lesions of white matter, hippocampal and cortical area. The spinal cord was thickened at THVIII - LI.

A de novo interstitial deletion of 1.3 Mb in 17q11.2 was detected at high-resolution oligonucleotide array CGH. By MLPA this deletion was found to be the most common NF1 microdeletion (type 1) spanning 1.4 Mb. This deletion is known to remove 14 genes. Some dysmorphic features, intestinal malrotation and some of the brain abnormalities have not been reported previously to be associated with NF1 microdeletion patients.

#### P02.137

##### Spinal neurofibromatosis- a rare *de novo* presentation of NF1

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The authors present a 16-year-old boy admitted to neurological department with clinical symptoms of polyneuropathy. He was born from first uneventful pregnancy and delivery, his psychomotor development was normal but frequently suffered from bronchitis. Family anamnesis without any history of inheritable diseases. When he was 11 the congenital heart defect (ASD) was diagnosed and successfully corrected. Few months after double operation of left hallux onyxis the steppage gait was observed. On the patient's skin two „café-au-lait” spots were noted as well as several neurofibromas along nerves in upper and lower limbs. Neurological examination and nerve conduction studies revealed neuropathy. MRI of the brain showed high intensity signal in globus pallidus on the right side. MRI of the vertebral canal and the spinal cord of cervical and lumbo-sacral regions revealed bilateral numerous neurofibromas comprising all the levels of nerve roots, along plexuses and nerve ways. In the cervical part neurofibromas invaginates into the vertebral canal with meningeal sack and spinal cord modeling. No other clinical symptoms of NF1 were found.

Spinal neurofibromatosis (OMIM #162210) is a rare manifestation of NF1 affecting nerve roots, occurring mostly in families. In most patients single or no diagnostic symptoms of NF1 have been noted. Molecular examinations made in some families confirmed mutations in neurofibromin gene. Presence of a gene modifying the clinical phenotype compensating for the deficiency of neurofibromine is suggested. As causal therapy is impossible, the only opportunity is symptomatic treatment.

#### P02.138

##### Phenotype- genotype correlations in Noonan/Leopard syndromes in a Portuguese Medical Genetics Department

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Noonan syndrome (NS) and LEOPARD syndrome (LS) are characterized by short stature, typical facies, cardiac anomalies (CA), haematological anomalies and skin involvement.

OBJECTIVE: Clinical and molecular characterization of patients and affected relatives with NS and LS.

MATERIAL AND METHODS: Patients with a clinical diagnosis of NS or LS were prospectively enrolled in this study conducted at 3 paediatric cardiology units. A clinical protocol was completed for all patients. A total of 104 patients, 84 index cases (IC) and 20 affected relatives (AR) were included. The known genes for NS/LS (PTPN11, KRAS, SOS1, and RAF1) were analysed by PCR amplification and sequencing.

RESULTS: Specific prenatal features were present in 18/84 (21%). Postnatally, 60/84 (71%) had short stature, 42/84 (50%) typical facies,

20/48 (42%) cryptorchidism. CA were present in 64/84 (76%) patients. Of these 30/64 (47%) had pulmonary valve stenosis, 8/64 (13%) atrial septal defect, 8/64 (13%) ventricular septal defect, 6/64 (9%) hypertrophic cardiomyopathy, 6/64 (9%) had stenosis of the peripheral pulmonary arteries and 9% others CA. Forty patients, 41% IC and 25% AR with CA were enrolled in the molecular genetic study. PTPN11 mutations are found in 22 patients, 4 patients had no detectable mutation and 14 are under molecular study. The PTPN11 mutation T468M was found in patients with either NS or LS. In two families the PTPN11 mutations, N308D and T468M were transmitted by fathers.

CONCLUSION: PTPN11 is the gene most frequently mutated in NS and LS in this cohort and in 2 families were transmitted by fathers.

#### P02.139

##### Germline BRAF mutations in Noonan, LEOPARD and cardiofaciocutaneous syndromes: molecular diversity and associated phenotypic spectrum

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Noonan, LEOPARD and cardiofaciocutaneous syndromes (NS, LS and CFCS) are developmental disorders with overlapping features including distinctive facial dysmorphia, reduced growth, cardiac defects, skeletal and ectodermal anomalies, and variable cognitive deficits. Dysregulated RAS-mitogen-activated protein kinase (MAPK) signal traffic has been established to represent the molecular pathogenic cause underlying these conditions. To investigate the phenotypic spectrum and molecular diversity of germline mutations affecting BRAF, which encodes a serine/threonine kinase functioning as a RAS effector frequently mutated in CFCS, subjects with a diagnosis of NS (N= 270), LS (N= 6) and CFCS (N= 33), and no mutation in PTPN11, SOS1, KRAS, RAF1, MEK1 or MEK2, were screened for the entire coding sequence of the gene. Besides the expected high prevalence of mutations observed among CFCS patients (52%), a *de novo* heterozygous missense change was identified in one subject with LS (17%) and 5 individuals with NS (1.9%). Mutations mapped to multiple protein domains and largely did not overlap with cancer-associated defects. NS-causing mutations had not been documented in CFCS, suggesting that the phenotypes arising from germline BRAF defects might be allele specific. Selected mutant BRAF proteins promoted variable gain of function of the kinase, but appeared less activating compared than the recurrent cancer-associated p.Val600Glu mutant. Our findings provide evidence for a wide phenotypic diversity associated with mutations affecting BRAF, and occurrence of a clinical continuum associated with these molecular lesions.

#### P02.140

##### Noonan Syndrome and Neurofibromatosis type I in a family with a novel mutation in NF1

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Noonan Syndrome (NS) and Neurofibromatosis type I (NF1) belong to a group of clinically related disorders that share a common pathogenesis, dysregulation of the RAS-MAPK pathway. NS is characterised by short stature, heart defect, pectus deformity and facial dysmorphism, while skin manifestations, skeletal defects, Lisch nodules and neurofibromas are characteristic of NF1. Both disorders display considerable clinical variability. Features of NS have been observed in individuals with NF1 - a condition known as Neurofibromatosis-Noonan Syndrome

(NFNS). The major gene causing NFNS is *NF1*. Rarely, a mutation in *PTPN11* in addition to an *NF1* mutation is present.

We present the clinical and molecular characterisation of a family displaying both NS and *NF1* features, with complete absence of neurofibromas. To investigate the aetiology of the phenotype, mutational analysis of *NF1* was conducted, revealing a novel missense mutation in exon 24, p.L1390F, affecting the GAP-domain. Additional RAS-MAPK pathway genes were examined, but no additional mutations were identified. We confirm that *NF1* mutations are involved in the aetiology of NFNS. Furthermore, based on our results and previous studies we suggest that evaluation of the GAP-domain of *NF1* should be prioritised in NFNS.

#### P02.141

##### Molecular and clinical characterization of 37 patients with Noonan syndrome

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Noonan syndrome (NS, OMIM 163950) is an autosomal dominant disorder, with a prevalence of 1:1000-1:2500 live births, characterized by short stature, facial and skeletal dysmorphisms, cardiovascular defects and haematological anomalies. Missense mutations of *PTPN11* gene account for approximately 50% of NS cases, while molecular lesions of other genes of the RAS/MAPK pathway play a minor role in the molecular pathogenesis of the disease. Twenty-nine sporadic and 4 familial cases of NS, for a total of 37 patients, underwent molecular analysis of the main genes of the pathway with a total mutation detection rate of 78.8% (26/33). In details, we found 15 sporadic and 2 familial *PTPN11* (51.5%), 6 sporadic and 1 familial *SOS1* (21.2%), 1 sporadic *KRAS* and 1 sporadic *BRAF* (3%) mutated cases. The two *PTPN11* familial cases were characterized by a very high intrafamilial variability, with a surprisingly mild facial phenotype. Interestingly, we have observed some peculiar clinical features in *SOS1* patients, in particular a prominent metopic suture in a turricephalyc cranial vault, not observed in *PTPN11* mutated NS patients. The *KRAS* patient presented typical NS dysmorphisms not associated with congenital heart defects, while the *BRAF* patient, in addition to the characteristic NS phenotype, presented epilepsy, a severe mental retardation and a Chiari type I malformation, so far reported only in 4 other cases; it is possible to propose that the cervical-occipital anomaly resulting in Chiari I malformation, and its variants, can be an aspect of the skeletal dysplasia of the syndrome.

#### P02.142

##### Transcriptional hallmarks of Noonan syndrome in peripheral blood mononuclear cells

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Noonan syndrome (NS) is an autosomal dominant syndrome characterized by a distinctive facial appearance, heart defects and skeletal abnormalities, rarely associated with mental retardation or juvenile myelomonocytic leukemia. The majority of germline mutations responsible for this disorder are in the *PTPN11* and *SOS1* genes encoding proteins of the Ras-MAPK pathway, which regulates cell proliferation, differentiation and senescence by controlling gene expression. To investigate the transcriptional consequences of these mutations, we performed Global mRNA Expression Profiling (GEP) with Illumina oligonucleotide microarrays on Peripheral Blood Mononuclear Cells (PBMCs), a target tissue of the syndrome. In detail, we analyzed 23 samples from molecularly defined NS patients (17 with *PTPN11* and 6 with *SOS1* mutation), and 20 samples from age- and sex-matched controls. Out of over 20,000 genes analyzed, 5,254 passed a statistical filter for reliable signal detection and for not being correlated with age, sex, or differential leucocyte count. Subsequently, t-test and signal-to-noise ratio were used to select genes differentially expressed between control samples and NS cases, all together or subdivided in *PTPN11* and *SOS1* subgroups. Interestingly, GEP analysis highlighted

a transcriptional profile specifically associated to the mutational status of NS samples. Both *PTPN11* and *SOS1* subgroups were well distinguished from control samples, however displaying clearly distinct patterns of gene expression, not consistent with a homogeneous generic NS group. These data provide initial evidence of a high potential for PBMCs GEP analysis to dissect at transcriptional level the molecular complexity of the inherited developmental disorders of the Ras-MAPK pathway.

#### P02.143

##### A possible role for the *PTPN11* gene in sex determination

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The *PTPN11* (protein tyrosine phosphatase, non-receptor type 11) gene carries the instructions for making a protein called protein tyrosine phosphatase, nonreceptor type 11, more commonly known as SHP-2. SHP2 forms a subgroup of this class and is a key player in generating signals within cells that affect cell function, division and differentiation. Germline mutations in this gene have been known to be associated with various diseases, including Noonan syndrome, LEOPARD syndrome, and juvenile myelomonocytic leukemia (JMML). The case presented here was diagnosed antenatally with cystic hygroma, hydrops fetalis, bilateral club feet, ASD and a karyotype of 46XX. Termination of pregnancy occurred at 17 weeks of gestation and autopsy identified ambiguous genitalia and normal testes. DNA analysis for *PTPN11* showed a heterozygous C > A nucleotide change in exon 13, which has not been previously reported. The mutation is found in a strongly conserved domain across species.

The spectrum of *PTPN11* mutations and their clinical implications are wide-spread, explained by the ubiquitous expression of this protein. It remains interesting that association of the gene with sex differentiation or ambiguous genitalia has not been made previously. Our patient was found to have a novel, likely pathogenic *PTPN11* mutation. Although the karyotype revealed a normal female genotype, the gonads were normal testes with the external genitalia showing ambiguity. The absence of SRY gene and the finding of a de novo, germline mutation in the *PTPN11* gene raises a possible important role for the *PTPN11* gene in sex determination.

#### P02.144

##### Mutation database of Noonan, Costello and cardio-facio-cutaneous (CFC) syndromes

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Noonan syndrome, Costello syndrome and cardio-facio-cutaneous (CFC) syndrome are autosomal dominant multiple congenital anomaly syndromes characterized by a distinctive facial appearance, heart defects, musculoskeletal abnormalities and mental retardation. There has been growing evidence that these syndromes are caused by dysregulation of the RAS/mitogen activated protein kinase (MAPK) pathway. Noonan syndrome is caused by mutations in *PTPN11*, *KRAS*, *SOS1*, or *RAF1*. Costello syndrome is caused by mutations in *HRAS*, whereas CFC syndrome is associated with mutations in *KRAS*, *BRAF* or *MAP2K1/2*. The molecules encoded by these genes in the pathway play pivotal roles in cell proliferation, differentiation, survival and cell death. We recently suggested that disorders with mutations of molecules in the RAS/MAPK cascade may be comprehensively termed "the RAS/MAPK syndromes" (Aoki et al., Hum Mutat 29(8):992-1006, 2008). As more mutations were identified in affected patients, significant overlaps of clinical pictures among these syndromes became evident. It may be necessary to re-classify these syndromes according to molecular diagnosis and re-evaluate clinical manifestations. To this end, we launched a website including the details on the MAPK syndromes and mutation database (<http://www.medgen.med.tohoku.ac.jp/RasMapk syndromes.html>). Although the database is still in its infancy, it would help us to overview the spectrum of mutations of various genes and to aid genetic testing of these elusive disorders.

## P02.145

**Noonan-syndrome-like phenotype associated with a familial 12q13 about 1.1 Mb microduplication proximal to the PTPN11-gene. RT-PCR-investigations on a large number of Noonan syndrome patients who were found to be negative for mutations in genes related to this disorder for similar genome aberrations.**

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Noonan-syndrome, a relatively common heterogeneous disorder (estimated incidence of 1 in 1000 - 2500 live births) was shown to occur due to dysregulation of the RAS-MAPK pathway. Gain-of-function mutations in the PTPN11, KRAS, SOS1, and RAF1 genes from the RAS/MEPK signalling pathway can be identified in about 70-80 % of individuals with Noonan syndrome. In 40-50 % mutations of the PTPN11 gene were found to be responsible with considerable phenotype variability. There is an overlap of phenotype features with Costello- and CFC-syndrome, also developmental disorders of the RAS-RAF-MAPK pathway. Recently an 8 Mb large duplication in 12q24 including the PTPN11 gene was reported in a Noonan syndrome patient, who was negative for mutations of the PTPN11, KRAS, SOS1 and RAF1 genes. The conclusion that duplication of the PTPN11 gene could be responsible was therefore put forward. Here we describe a new microduplication of about 1.1 Mb at 12q13 identified by microarray analysis and verified by RT-PCR in a 7 year old boy and his mother who both show physical including facial and mental symptoms compatible with diagnosis of Noonan syndrome. Sequencing analysis of the PTPN11 and KRAS genes did not show a causative mutation. Interestingly a gene of the MAP-kinase family is duplicated in these patients. Results of a detailed investigation for genome copy alterations by RT-PCR-analysis using primer sets for the proximal, central and distal part of this 1.1 Mb microduplication in more than 100 Noonan syndrome patients negative for mutations in Noonan syndrome related genes will be presented.

## P02.146

**Molecular analysis of the Noonan (-like) Syndromes: overview of 7 years of DNA diagnostics in the Netherlands**

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The clinically related Noonan syndrome, Cardio-Facio-Cutaneous (CFC) syndrome, and Costello syndrome are characterised by a typical facial appearance with hypertelorism, ptosis, down slanting palpebral fissures, low set posteriorly rotated ears, and short neck. Other characteristic findings include congenital heart disease, mental retardation, and short stature. These disorders are caused by dysregulation of the RAS/mitogen activated protein kinase (MAPK) pathway, due to mutations in the genes PTPN11, SOS1, RAF1 (Noonan syndrome), BRAF, MAP2K1, MAP2K2 (CFC syndrome), HRAS (Costello syndrome), or KRAS (Noonan, CFC, and Costello syndrome).

In our laboratory, PTPN11 mutation analysis has been performed in more than 1000 index cases with a clinical suspicion of Noonan/CFC/Costello syndrome. In 25% of patients a mutation could be identified, up to 80% of which appeared to be *de novo*. In one family, homozygosity for a PTPN11 mutation caused severe cardiac disease in two fetuses, and lethality shortly after birth. Selective mutation analysis of the other genes in the MAPK pathway led to a molecular diagnosis in an additional 30% of patients.

The recent implementation of a high-throughput automated parallel sequencing protocol of all known genes involved in Noonan/CFC/Costello syndrome in our laboratory, has led to the identification of novel mutations. We gained more insight in genotype/phenotype correlations, and concluded that more Noonan (-like) genes remain to be discovered. Furthermore, there is an urgent need for a prenatal testing protocol, since we identified one PTPN11 and one KRAS mutation in fetuses with polyhydramnion and increased nuchal fluid with a normal karyotype.

## P02.147

**Atypical molecular findings in four patients with suggestive Noonan-related Syndromes**

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Germline missense mutations in genes coding for different components of the RAS/MAPK signalling cascade (PTPN11, SOS1, RAF1 and RAS) have been recognized as the cause of several phenotypically overlapping autosomal-dominant disorders, recently referred to as the Noonan-related Syndromes (Noonan, LEOPARD, Costello and Cardiofaciocutaneous syndromes). Herein we describe four patients presenting atypical findings in molecular diagnosis.

Patient #351 had suggestive facial dysmorphology, severe short stature and bilateral cryptorchidism. DNA testing identified an in-frame 3pb-insertion in exon-7 of PTPN11 gene (p.Q255\_Q256insQ). Two other affected members of the family segregate the variant.

Patient #178 presented with facial dysmorphology and mild short stature. PTPN11 assays were performed, revealing a 3pb-deletion in intron-12 (c.-16\_-18delATG). In silico splicing simulation produced new acceptor splice sites, thus probably affecting the correct splicing.

Patient #301 displayed craniofacial anomalies with severe short stature, pulmonic stenosis and hypertrophic cardiomyopathy, moderate developmental delay, and severe linfatic dysplasia. PTPN11 gene analysis revealed the presence of a *de novo* single-codon double-nucleotide substitution (p.T73L) that produces an aminoacidic change not previously reported.

Patient #255 had typical facial phenotype, pulmonic stenosis, hypertrophic cardiomyopathy and severe feeding difficulties. Molecular diagnosis showed a heterozygous 6pb-deletion in intron-1 5'UTR region in HRAS gene (c.-84\_-89delGGGCCT). Mutations in 5'UTR might affect regulation of gene expression.

This study describes four patients with suggestive Noonan-related Syndromes with atypical molecular findings. Phenotypical consequences of all these mutations have not been proved with an *in vitro* analysis, but some indirect approaches have been performed to explore the effect of these atypical variants.

## P02.148

**Duplication of the Rubinstein-Taybi locus at 16p13: a novel case with overgrowth is broadening the phenotypic spectrum**

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Microduplications encompassing the Rubinstein-Taybi region at 16p13.3 lead to a rare multiple congenital anomalies/ mental retardation syndrome. We report on a 19 years old male patient with an overgrowth syndrome with multiple congenital anomalies and mild mental retardation due to a 0.95 Mb duplication at 16p13.3. He presented with a left dysplastic ear with atresia of the external auditory canal, a preauricular tag, fused middle ear ossicles and bilateral deafness. The dysmorphic facial features were characteristic for a long and narrow face, deep set eyes with low position of eyebrows, prominent nasal root and tip, lateral flaring of eyebrows, short philtrum, prominent lips, everted upper lip and large hand and feet with broad interdigital joints. He showed tall stature with a mild scoliosis and a marked thoracic kyphosis. Beside the typical facial and skeletal features, overgrowth seems to be a key feature for submicroscopic duplications of the Rubinstein-Taybi region. Many of the clinical features of our patient fit well with the three cases published so far; however, some are unique (in addition, he presented ear anomalies, very distinctly advanced growth and relatively mild developmental delay), delineating the common clinical phenotype on one side and broadening the clinical spectrum on the other side. From this observation, we conclude that the evaluation of patients with overgrowth syndromes with variable degree of mental retardation should also include the investigation for submicroscopic chromosomal imbalances.

## P02.149

## A chest tumor in a child with Rubinstein Taybi syndrome - Case report

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Background : Rubinstein Taybi is a rare syndrome caused by genetic defect.

Matherial and method: D.I, 4 years old, female patient, with particular phenotype, admitted in our hospital in may 2004, for polyarticular arthritis. She followed clinical examination, and analysis (blood count, ESR, rheumatoid factor, immunoglobulins, karyotype, bacteriological analysis from blood, throat, nose, urine), NPI, ophthalmological and x-ray exams, MRI. was perform.

Results and discusions: The child was diagnosed with Rubinstein-Taybi syndrome [by facial abnormalities, specific digital abnormalities, growth retardation (> 3 SD) and mental retardation, genetic defect on 16p13] and oligoarthritis negative RF. Under steroid therapy the arthritis evolution was well but the child get a right paracardiac pneumonia - treated 13 days with third generation cephalosporins. The X-ray control exam shows in a right paracardiac a small tumor (2/2 cm) confirmed by MRI. The pathological exam indicates a paravertebral rhabdomyosaroma. The girl was transferred in the oncology department and received the standard protocols. The evolution was good she is in the second grade at school, and there are no malign relapse signs.

Conclusions : • The Rubinstein Taybi syndrome is rare;  
• The diagnosis is made by clinical exams and with association of the high quality (pro) metaphase genetic exams;  
• The association of this syndrome with right paravertebral rhabdomyosaroma and paraneoplastic arthritis is rare.  
• There is a great need to have more sophisticated methods for investigating this syndrome in our department.

## P02.150

## A series of patients with Rubinstein-Taybi syndrome: Review of clinical features

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Cerrahpasa Medical Faculty, Department of Medical Genetics, Istanbul, Turkey. Rubinstein Taybi Syndrome (RTS) is a rare disorder, characterised by distinctive facial features, broad and often angulated thumbs and great toes, short stature, and moderate to severe mental retardation. Other frequent symptoms include seizures, hypotonia, hyperreflexia, microcephaly, frontal bossing, down-slanting palpebral fissures, small mouth, frontal hair-up sweep, ear abnormalities, micrognathia, heavy eyebrows, long eyelashes, ptosis, epicanthal folds, strabismus, persistent fetal finger pads, scoliosis, spina bifida occulta, cryptorchidism, hirsutism, and capillary hemangioma. Cardiac defects and structural brain abnormalities have also been occasionally reported. Although RTS is inherited in an autosomal dominant manner, and clinical variability is common in this mode of inheritance, we did not observe a remarkable clinical heterogeneity among our series of patients. Here, we report the clinical features of five RTS patients and compare them with the anomalies reported in the literature. Mental retardation, hypotonia, down-slanting palpebral fissures, small opening of mouth, hypoplastic alae nasi, ear abnormalities, broad toe and thumbs were evident in all patients, supporting the previously reported high ratios of these findings. Additionally, optic disc pallor and chorioretinal atrophy were detected in one of the present cases. We suspect that these are novel findings, which have not been described in the clinical picture of RTS up to date.

## P02.151

## Recurrence of achondrogenesis type II within the same family

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Achondrogenesis type II (ACG2) is a lethal dysplastic disorder of the skeleton caused in most cases by a novel dominant mutation in the type II collagen gene (COL2A1) which is present in the heterozygous state. ACG2 is characterized micromelia, absent of ossification in the vertebral column, sacrum and pubic bones. The trunk is short with prominent abdomen and hydroptic appearance.

We report a family where consulted patient had had severe scoliosis, body disproportion with short trunk. Her first pregnancy was termi-

nated due to severe pathological findings noted on fetal ultrasound investigation. Clinical picture and radiological investigations of the fetus showed typical findings of achondrogenesis type II. Molecular analysis of genomic DNA extracted from fetal cells (Prof. Geert Mortier, Ghent University) revealed the heterozygosity for c.2473G>T mutation (p.Gly825Arg) in the COL2A1 gene.

Ultrasound investigations during the second pregnancy revealed fetal hygroma, severe micromelia suggesting the recurrence of achondrogenesis type II within this family.

Clinical and radiological findings in mother are not similar to the skeletal anomalies present in children.

As the family history is complicated with the disorder which affects the same organ system in the mother and siblings we suspect that the different and milder phenotype in mother is due to the somatic mosaicism of the mutant COL2A1 gene. The molecular analyses of the parents DNA are in process in the time of abstract submission.

## P02.152

## Acro-mandibulofacial dysostosis associated with microcephaly and mental retardation: a new case

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Acro-mandibulofacial dysostosis is a rare and heterogeneous group of conditions, characterized by variable craniofacial features including malar hypoplasia, micrognathia, external ears defects and limb abnormalities. Among them, Nager syndrome (preaxial acrofacial dysostosis) is the most common. Post-axial acrofacial dysostoses include close entities such as Miller, Wildervanck-Smith, and Genée-Wiedemann syndromes. Mandibulofacial dysostosis may be also associated with slit-foot deformity.

In 2000 and 2006, four Brazilian patients were reported with a characteristic combination of mandibulofacial dysostosis associated with marked microcephaly, trigonocephaly, preauricular skin tags, cleft palate and mental retardation. Preaxial polydactyly was observed in two cases. All cases showed feeding difficulties, post natal growth retardation, and severe delay in language development.

We report a further patient with the same association of mandibulofacial dysostosis, pre and post-natal microcephaly on -4 SD, preauricular skin tags, large ear lobes and abnormal thumbs. The patient, the first child of unrelated healthy Caribbean parents, also presented with severe feeding difficulties and severe delay in language development.

In conjunction with the other reported Brazilian cases, our observation gives support to a novel original phenotype within the complex group of mandibulofacial dysostosis. The molecular bases of this condition remains unknown, as genetics investigations, including CGH arrays in our patient, were normal. Hitherto, all cases are sporadic and an autosomal recessive inheritance may be considered based on parental consanguinity in one Brazilian case; however, *de novo* dominant mutation at an autosomal locus cannot be excluded.

## P02.153

Larsen Syndrome: clinical report of a patient with a new *de novo* mutation

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**Introduction:** Larsen *et al.* (1950) described one of the original cases of this bone dysplasia in a patient that in 1972 had an affected child. Features of Larsen syndrome (MIM: #150250) are dislocations of large joints and unusual facies. Characteristic radiologic findings are also present. Larsen syndrome is inherited in an autosomal dominant manner. It's a rare disease, around 100 cases have been published. In 2004 *de novo* missense mutations in the FLNB gene were described. Here we report a new heterozygous *de novo* mutation in a patient with Larsen syndrome.

**Case Report:** First and only child of non-consanguineous parents. At birth, he presented with congenital anomalies: cleft palate, bilateral clubfoot, camptodactyly and arthrogryposis.

Referred to our Genetics consultation at the age of four. Physical examination showed knee and elbow dislocation, arthrogryposis, kyphoscoliosis and spatulate fingers with broad thumbs and halluces. Face was flat with prominent forehead and short nose with flat nasal bridge. Psychomotor development was normal.

Skeletal radiography showed rhizomelia of upper and lower limbs and multiple joint dislocations. Anterior decompression and posterior fusion was attempted at the age of 5.

The molecular study was performed after informed consent within a research environment. The mutation 4580T>C was found, leading to an aminoacid substitution at position 1527 of the Filamin B protein.

**Discussion:** The mutation 4580T>C had not been described before. Examination of parental samples showed that this mutation has occurred *de novo*. Additional investigation is ongoing to further characterize this new mutation, including functional studies in fibroblasts.

#### P02.154

##### “Laurin-Sandrow Syndrome”: a new family with rare genetic disorder

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“Laurin-Sandrow syndrome” (LSS) (OMIM 135750) is rare disorder characterized by distinct combination of mirror polysyndactyly, symmetric tetramelia, nasal defects.

We described a new family with autosomal dominant transmission of LSS, presented a clinical findings of affected child and father, reviewed a literature data according diagnostic criteria.

Proposita - second child of young couple (G1- healthy sister) was born at term BW=2110; BL=42cm; OFC=32 cm. Female infant showed prenatal hypoplasia, microcephaly, posterior encephalocele, dysmorphic ears, facial appearance similar to frontonasal dysplasia (hypertelorism, microphthalmia, partial aplasia of nasal bones, partial atresia of nasal canals, wide flat nose, nares defects, unilateral cleft lip and palate, micrognathia), heart defect (ASD), bilateral mesomelic anomalies of lower limbs, pre- and postaxial polydactyly of feet (8 toes), syndactyly, clubfoot. At follow-up growth and mental delay, neurological signs, pyeloectasia were developed. Child died at 3 months age.

Father presented normal limbs, growth (W-63kg, L-172cm, OFC-55cm), mental development, unusual facial features - small soft mass (no biopsy) on the left part of forehead, hypertelorism (40 mm), ectopia of lacrimal ducts, myopia, astigmatism, angiopathia of retina, wide flat nasal bridge, abnormal nostrils (nasal defects were operated at 18 years old). Echocardiography: additional chordae of left ventricle. Karyotype: 46, XY. His parents, sister, nephew showed normal phenotype.

Clinical features of our patients were compared with published data. We diagnosed “Laurin-Sandrow syndrome” based on association of characteristic signs - mirror polysyndactyly, bilateral mesomelic limbs, nasal defects. Presented family illustrated a wide variability of phenotype's spectrum and confirmed autosomal dominant inheritance of LSS.

#### P02.155

##### Array-CGH analysis in a series of 54 index patients with limb malformation identified more than 10% anomalies

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Congenital limb malformations (CLM) affect approximately 1 in 500 new-borns. They can be isolated or part of multiple congenital anomalies (MCA) syndromes and are due to two major causes: intrauterine

disruptions (ID) and genetic abnormalities. The second group is very heterogeneous and, although many chromosomal abnormalities and genomic alterations have been described, most of CLM remain unexplained.

We performed array-CGH (Agilent 244K) in a series of 54 index patients with normal karyotype and unexplained CLM either isolated (10) or part of known or unknown MCA syndromes (44). In 19 patients, the gene responsible for the disease had been previously unsuccessfully tested. In the remaining 35 patients, no gene testing was available or a precise clinical diagnosis could not be achieved.

Thirty analyses detected either no genomic variation or known CNVs (55%). In 5 index patients, array-CGH revealed one or more unknown CNVs which either were present in one asymptomatic parent (4) or did not segregate with the malformation (1). We identified anomalies in the remaining 19 index patients. Three of them were deleterious, either *de novo* (1) or familial (2). One recurrent identical anomaly identified in 3 index patients sharing similar radial malformations could be deleterious, but familial additional analyses are still pending for these and the other 13.

Overall, at least 6 genomic anomalies have been identified in this series of 54 patients presenting limb malformations ( $\geq 11\%$ ).

**Conclusion:** After precise clinical and genetic evaluation, unexplained CLM are good candidates for Array-CGH analysis.

#### P02.156

##### A Study of the Genetics of Limb Reduction Defects

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Limb reduction defects are an important group of congenital limb malformations that requires thorough assessment. They can be isolated or associated with other malformations as a part of syndrome. Causes of limb deficiencies include single gene disorders, chromosomal abnormalities or teratogens. However, the etiology remains unknown in many cases. The present study aimed at the proper diagnosis and classification of 30 cases with limb defects referred to the Limb Malformations and Skeletal Dysplasia Clinic, NRC, Egypt in order to provide accurate and efficient genetic counseling. Detailed history, three generation pedigree analysis, examination of different body systems with specific studies of different parts of the limbs documented by radiological examination, photography and basic anthropometric measurements were conducted for all cases. Dermatoglyphic analysis, cytogenetic studies and other investigations were done whenever indicated. Cases were classified according to Temtamy and McKusick (1978) into 8 groups; isolated terminal transverse defects (6/30), terminal transverse defect as a part of syndrome (2/30), isolated radial defect (1/30), radial defect as a part of syndrome (9/30), isolated ulnar defect (2/30), ulnar defect as a part of syndrome (6/30), pre and postaxial defect (1/30) and axial defect as a part of syndrome (3/30). The results of this study have shown that limb absence or reduction defects are not an uncommon malformation among Egyptian children. Delineation of the exact cause, correct classification and diagnosis are essential for proper genetic counselling. Molecular studies are recommended for accurate diagnosis and understanding of the pathogenesis.

#### P02.157

##### Autosomal-recessive primary hypertrophic osteoarthropathy due to mutations in the 15-OH-prostaglandin dehydrogenase gene: 15- year follow-up in two Austrian siblings

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**Background:** Hypertrophic osteoarthropathy (HO) is commonly associated with clubbed fingers as a consequence of cardiopulmonary disease. In contrast, primary forms (PHO) are rare familial disorders which may show dominant or recessive inheritance. Features may include skin thickening (usually referred to as pachydermoperiostosis) cranial vault abnormalities and patent ductus arteriosus. Recently, the pathogenesis of these disorders was shown to be related to mutations of the 15-OH-prostaglandin dehydrogenase (HPGD) gene.

**Clinical report.** We report on the clinical course of Austrian sibs affect-

ed with the recessive form of this condition: they have clubbed fingers, painful periostitis, excessive sweating on hands and feet, thickening of the limbs particularly knees with arthralgia of large and small joints, and paroxysmally occurring bone pains. All these symptoms started after the first few years of life. Radiologically, cortical thickening and sclerosis of the tubular bones with expansion of the diaphyses, and Wormian bones of the skull were shown. The bone symptoms grossly faded away - however pain attacks and hyperhidrosis still persist. Molecular analysis demonstrated the presence of frameshift mutations of both HPGD alleles: c.120delA (paternal) and c.175\_176 del-CT (maternal). The heterozygous parents are healthy. Investigation of the presumably increased PGE2-excretion is under way.

**Conclusion.** Given the causality between the painful clinical symptoms and disturbance of the prostaglandine metabolism, antagonist drugs like indomethacin may alleviate clinical symptoms, using PGE2-excretion as a useful monitoring of this therapy.

#### P02.158

#### Blomstrand dysplasia - evidence of founder effect in a small northeast Brazilian region

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Blomstrand Dysplasia (BD), a lethal osteochondrodysplasia (OCD), is characterized by osteosclerosis, carpo-tarsal advanced maturation, narrow thorax with clavicles and ribs thickening, and short limbs displaying a dumb-bell appearance of the tubular bones. Other features are hydrops, macroglossia, and atelia. Recessive inheritance was recently confirmed by the description of homozygous mutations in the PTHR1 gene. In this report we present a study of two new families coming from three near small cities of the Brazilian northeast region. Consanguinity was not referred by the parents of the first studied family and the proband's clinical-radiological natal examination revealed a typical phenotype of BD. In the other studied family, the parents are consanguineous and the proband's radiological-clinical evaluation confirmed the BD diagnosis. In both probands an identical single homozygous point mutation at position -2 of the 5' consensus acceptor site at the exon 9 (c.639 -2 A>G) was identified after PCR amplification of all coding exons and intron-exon junctions of the PTHR1 gene. The point mutation was present at the heterozygous state in the proband 1's parents. This mutation is expected to result in a loss of function of the PTHR1. On the fifteen BD cases previously reported, one was exactly from the same region referred for the families here reported. In conclusion, considering that three families have segregated this rare condition come from the same region and the fact that in two of the probands were found the same mutation, we suggest a founder effect in BD at the Brazilian northeast region.

#### P02.159

#### Molecular diagnosis in Pycnodysostosis

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**Introduction:** Pycnodysostosis is a rare autosomal recessive trait, characterized by osteosclerosis, short stature, acro-osteolysis of the distal phalanges, bone fragility, clavicular dysplasia, and skull deformities with delayed suture closure. The responsible gene, cathepsin K (CTSK), is a lysosomal cysteine proteinase critical for bone remodeling and reabsorption. CTSK spans approximately 12 kb in 1q21 and contains 8 exons. Its 987bp open reading frame encodes a polypeptide of 329 amino acids. CGC Genetics is a reference laboratory for diagnosing this disease. We report here our experience for the identification of new Pycnodysostosis cases.

**Method:** During 2004-2008, we received 9 samples for CTSK gene analysis. Our approach is the sequence analysis of the complete coding region of this gene including adjacent intron/exon boundaries. At this moment we designed a microarray based assay (patent pending), to be used as a first evaluation that detects 15 mutations and includes those referred in the present work.

**Results:** In 5 patients we found 1 homozygous disease causing mutation plus 2 new mutations (1 nonsense and 1 frameshift). One patient with 1 causative mutation and 1 new amino acid change, and 1 patient with 1 new homozygous amino acid change. In the remaining 2

patients, no mutation was identified. The newly detected amino acid change, in two cases, is predicted to be damaging.

**Conclusion:** These results, with four new mutations identified in unrelated families, emphasize the molecular heterogeneity of the defects in CTSK gene in Pycnodysostosis and the importance of genotyping for diagnosis and genetic counseling.

#### P02.160

#### Type 3 Rhizomelic chondrodysplasia punctata in a patient: A case report of a very rare disorder

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Rhizomelic chondrodysplasia punctata (RCDP) is an autosomal recessive disorder, characterized by severe limb shortening, punctate calcification of cartilage, flexion contractures, vertebral clefts, cataracts, characteristic facial appearance, severe growth deficiency and mental retardation. Three genotypes have been identified. Type 1 is associated with a defect in the PEX7 gene, which codes for the receptor of peroxisome targeting sequence 2. In Type 2, there is an isolated deficiency of DHAP acyltransferase, and in Type 3, a defect of alkyl-DHAP synthase. Plasmalogen levels are reduced in type 2 and 3 patients, while phytanic acid levels and the processing of 3-ketothiolase are normal. Here, we present a 3<sup>5/12</sup> years old patient, born to first cousin parents. He was first referred to our clinic, when he was 5 months old, because of growth delay, rhizomelic shortening of limbs, bilateral cataracts, and facial dysmorphism. His physical examination revealed that he had severe short stature, large anterior fontanel, microcephaly, high sloping forehead, micrognathia, bilateral cataracts, low and broad nasal bridge, anteverted nostrils, contractures, osteopenia, irregular vertebral endplates, rhizomelic shortening of all limbs. Skeletal radiologic studies displayed punctate calcifications on a number of cartilages. Biochemical investigations of fibroblasts revealed deficient alkyl-DHAP synthase. In conclusion, based on clinical picture and biochemical enzyme assay the diagnosis of Rhizomelic chondrodysplasia punctata type 3, which is an extremely rare disorder, has been diagnosed in this patient.

#### P02.161

#### A new classification system for segmentation defects of the vertebrae, and pilot validation study

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The study aimed to develop and assess a new classification system for Segmentation Defects of the Vertebrae (SDV), a frequent cause of congenital scoliosis. Existing nomenclature for the wide range of SDV phenotypes is inadequate and confusing, eg 'Jarcho-Levin syndrome'. A multidisciplinary group of ICVAS met to formulate a clinically useful classification, based primarily on radiology, and transferable to bioinformatic approaches. SDV are identified by number affected, contiguity, and spinal region(s). The size, shape and symmetry of the thoracic cage, and rib number, symmetry and fusion are included, and familiar vertebral morphology terms retained, together with accepted syndrome names. The terms *spondylocostal* and *spondylothoracic dysostosis* apply only to phenotypes typified by the monogenic disorders due to mutated *DLL3*, *MESP2*, *LNFG* and *HES7* genes. Five ICVAS members (Group 1) then independently assessed 10 new cases, inter-observer reliability assessed using kappa. Seven independent radiologists (Group 2) then assessed the same cases before and after introduction to the new system. Inter-observer reliability for Group 1 yielded a kappa value of 0.21 (95% confidence intervals (CI) 0.052, 0.366, p=0.0046). For Group 2, before introduction to the new system, 1/70 responses (1.4%) agreed with the Group 1 consensus, 12 differ-

ent diagnoses were offered, and 38/70 (54.3%) responses were 'Unknown'. After introduction to the new system 47/70 responses (67.1%; 95% CI 55.5, 77.0) agreed with Group 1 consensus, a 65.7% improvement (95% CI 52.5, 75.6,  $p<0.00005$ ). The system was well received by 6/7 radiologists. The new system was found to be reliable and acceptable.

#### P02.162

##### Opsismodysplasia in a newborn: Case report of a spondyloepimetaphyseal dysplasia

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Opsismodysplasia, a spondyloepimetaphyseal dysplasia is caused by a defect of chondroosseous transformation, with only 40 published cases. Inheritance is thought to be autosomal recessive, no causative gene has yet been identified. It is characterized by consistent clinical signs including craniofacial abnormalities, rhizomelic micromelia and muscular hypotonia. The diagnosis is based on specific radiological findings. Clinical outcome is variable, depending on the severity of the phenotype, however neonatal lethality is common.

We report on a term boy, first-born to a healthy, non-consanguine couple. Prenatal ultrasound at 30 weeks of gestation demonstrated shortening of both femora. At birth, he presented with reduced muscle tone and dysmorphic features such as frontal bossing, broad and depressed nasal bridge, short nose with anteverted nostrils, long philtrum, hypertelorism, low set ears, and large fontanelles. Limbs were short, in particular hands and feet with narrowed chest. Weight and length were both  $<3$  percentile. Skeletal survey revealed characteristic findings for Opsismodysplasia: major delay in epiphyseal ossification, shortening of tubular bones, especially those of metacarpals, metatarsals and phalanges, platyspondyly, square shaped iliac bones with lateral and medial spurs. Severe muscular hypotonia caused feeding problems and necessitated nasogastral tube feeding. Although the boy showed impressive thoracic narrowness, he was normopnoeic. The further clinical course so far was uneventful.

Opsismodysplasia is a rare skeletal dysplasia. Awareness of this disorder and documentation of the clinical follow up is of utmost importance to help understand its natural course. More data is needed to better counsel parents regarding recurrence risk, prenatal diagnosis and prognosis.

#### P02.163

##### Results of the Polish 3-year population surveillance program for Smith-Lemli-Opitz syndrome: Are so many patients missed or misdiagnosed?

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Smith-Lemli-Opitz syndrome (SLOS) is a malformation disorder, in which an inborn error of cholesterol biosynthesis results in congenital anomalies and mental deficits. There is evidence that cholesterol supplementation improves the clinical course of SLOS. Since atypical and severe cases are frequently misdiagnosed, a proper diagnosis followed by appropriate therapy and reliable genetic counseling may be delayed. Underdiagnosing is the reason why data on the true incidence of the disease is limited. The results of our previous newborn screening, based on the carrier frequency of the two most common SLOS-causing mutations in Poland (p.W151X and p.V326L), would make SLOS one of the most frequent recessive disorders in our coun-

try (with an incidence of 1 : 2 300 - 1 : 3 937). This prompted us to carry out the study presented herein. The incidence and prevalence of Smith-Lemli-Opitz based on 3-year population surveillance were estimated as 1 in 83 168 and 1 in 866 273, respectively. The notable discrepancy between our previous carrier newborn screening and prospective results suggests that the syndrome is probably either misdiagnosed (underascertainment of mild and atypical cases) or results mainly in intrauterine and neonatal death. The effects of the causative SLOS mutations identified in Polish patients, especially of the *null* mutation p.W151X, and the data collected in our surveillance strongly suggest that SLOS embryos are lost very early and that the disease is responsible for a high number of first-trimester miscarriages. The study was supported by State Committee for Scientific Research PBZ-KBN-122/P05/01-10.

#### P02.164

##### Diaphanospondylodysostosis - another evidence for autosomal recessive inheritance and exclusion of *IFT80* gene

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Diaphanospondylodysostosis (DSD) is a newly described spinal dysostoses characterized by abnormal radiolucency of the spine, and typical rib gaps associated with renal cystic lesions. On the fifteen previously reported cases recurrence was found in four families and parental consanguinity in just one. Due to phenotypic similarities of DSD to Pax1 and Meox1 deficient mice the PAX1 and MEOX1 were recently studied as candidate genes, but both were excluded. In this report we relate a baby from consanguineous parents presenting new findings. The proband is a male infant (46,XY), born at 36 weeks and shortly dead. He presented the following features: Potter facies, low set ears, short neck, voluminous abdomen, hypotrophy of the lower limbs, club feet, hypoplastic buttocks, pulmonary hypoplasia, complex heart defect, multiple renal cysts around areas of immature blastema, and typical vertebrae segmentation defect with absence of ossification of vertebral bodies and pedicles with "zipper-like" appearance associated with rib's small gaps or absence. Other radiologic findings were: narrow pelvis with peculiar iliac wings as well as the ischiopubic rami, bilateral slender femur and club feet. Based on the similar renal manifestations observed in Jeune thoracic dysplasia, we also excluded *IFT80* as the causative gene by direct sequencing. On going studies will hopefully lead to the identification of the molecular basis of this rare and severe condition.

#### P02.165

##### SPONASTRIME Dysplasia- short stature with short dental roots and cataracts -a further case

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SPONASTRIME Dysplasia (SD) (OMIM#271510) is an autosomal recessive skeletal dysplasia of the spondyloepimetaphyseal dysplasia (SEMD). The name was derived from "spondylar and nasal alterations with striated metaphyses".

We report on a 14 years old girl with SD, short dental roots and cataracts. The girl was born after a normal pregnancy with discordant twins. She was the first twin and small for gestational age BW 2090g, L 43cm, OFC 31,5cm ( $<P10$ ), whereas the second twin was appropriate. During early childhood she developed bilateral cataracts, which were treated surgically. Her permanent teeth were small and had extremely short roots, which limited orthodontic treatment. Midfacial dysplasia with depressed nasal root, short upturned nose and short stature were present. At the age of 14 years her height was 142 cm (-2,12 SD), weight was 45kg (P25-50) and intelligence was normal. The typical radiological abnormalities consisted of platyspondyly, striations in the metaphyseal margin and flattened capital femoral epiphyses. The genetic defect is not known in SD and may be heterogenous. The patients with physical findings typical for SD, short dental roots and bilateral childhood cataracts represent a rare, but very distinct entity. Up to now 16 cases have been published.

## P02.166

**Novel mutation in SEDL gene in three sibling of Russian family with X-linked spondyloepiphyseal dysplasia tarda**

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Spondyloepiphyseal dysplasia tarda (SEDL; MIM 313400) is an X-linked recessive osteochondrodysplasia that occurs in approximately two of every one million people. This progressive skeletal disorder which manifests in childhood is characterized by disproportionate short stature with short neck and trunk, barrel chest and absence of systemic complications. Distinctive radiological signs are platyspondyly with hump-shaped central and posterior portions, narrow disc spaces, and mild to moderate epiphyseal dysplasia. The latter usually leads to premature secondary osteoarthritis often requiring hip arthroplasty. The disorder is caused by the mutations in SEDL gene located on Xp22.12-p22.31. SEDL encodes a 140 amino acid protein with a putative role in endoplasmic reticulum (ER)-to-Golgi vesicular transport.

Here we report Russian family case with three affected sibs of five sibs born in two mother wedlock. Two patients were investigated at the age of 19 and 26 years and had typical clinical and radiological signs of SEDL. Onset of the disease was at the age after of 10 with growth impairment, back pain and waddling gait. Osteoarthritic changes were present in both hip joints with flattening of the femoral heads. In the patients, the mutation analysis in SEDL gene was performed, and a novel hemizygous mutation c.133G>C leading to the amino acid substitution Ala45Pro was found. This mutation was not identified in healthy mother and in 88 control chromosomes (in 35 males and 24 females).

## P02.167

**TAR Syndrome Case Report**

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Extremely rare affection in current medical praxis, TAR syndrome represents an association of malformations that includes almost every tissues and organs: hematological, cardiac, gastro-intestinal, skeletal. Material and Method: A case of a newborn was presented, who was been hospitalized at 10 hours age with a complex malformed syndrome.

Results: The physical examination and the paraclinical investigations revealed us TAR syndrome. The historical record of the newborn was not containing any special/unusual elements: newborn female with the gestational age of 38 weeks, birth weight: 2750 g, natural born in cephalic presentation, APGAR score: 8/9, green amniotic fluid.

The physical examination was showing: pale tegument, purple items over the chest, short forearms, simian line, ogival mouth palate, low-implanted ears. The cardiologic examination revealed a moderate cardiomegaly with atrial septal defect and minimal left-right bridge.

The skeletal X-ray confirmed the presence of one malformation: short forearms with the congenital bilateral radial aplasia.

The head ultrasonography in dynamics showed the presence of this cystic formations specific for periventricularleucomalacia - cystic form. A second degree intraventricular haemorrhage was associated.

Thrombocytopenia was severe: initially 14000/mmc until 35-40000/mmc required several transfusions with thrombocyte concentrate.

The evolution was slow, but favorably under the treatment; the discharge was done with weekly monitoring of hemogram condition.

Conclusions:

- Specific signs were present, except gastro-intestinal affection - the patient didn't present lactose intolerance;
- The severe thrombocytopenia was maintained throughout hospitalization, but this didn't aggravate the intraventricular haemorrhage (this particular one had a relatively good evolution).

## P02.168

**Expression and functional analysis of *EFNB1* mutations in craniofrontonasal syndrome**

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<sup>1</sup>Institut für Humangenetik, Magdeburg, Germany, <sup>2</sup>Institut für Medizinische Genetik, Zürich, Switzerland, <sup>3</sup>Institut für Humangenetik, Münster, Germany. *EFNB1* gene (OMIM 300035) encodes ephrin-B1 - a member of the ephrin-receptor ligand family. Together with their receptors (Eph) ephrins form signalling complexes involved in cell adhesion and migration. Mutations of *EFNB1* cause craniofrontonasal syndrome (CFNS; OMIM 304110). We describe missense mutations c.161C>T (p.P54L) and c.332C>T (p.T111I), splice-site mutation c.406+2T>C and frameshift mutation c.614\_615delCT that were found in CFNS female patients. The splice site mutation activates a cryptic splice site resulting in a smaller transcript. The frameshift mutation causes a premature termination codon, but escapes nonsense-mediated mRNA decay. No truncated ephrin-B1 was detected by Western blot analysis in protein extracts from patient fibroblasts for both of these mutations. Absence of mutant protein shows that c.406+2T>C and c.614\_615delCT probably have loss-of-function effects. Both missense mutations lead to an amino acid exchange in the ephrin-B1 extracellular domain. In order to evaluate the impact of these mutations on signalling and cell behaviour, NIH 3T3 cells were cotransfected with pEGFP-N3 vector and pcDNA 3.1 (+) vector containing cDNA of wild-type or mutant type c.161C>T and c.332C>T *EFNB1*. Transfected cells were stimulated with EphB2-Fc/Fc and analysed using fluorescent microscopy. After stimulation wt- and p.T111I-ephrin-B1 expressing cells formed cell patches, whereas no patches were observed for p.P54L. Western blot analysis using anti-ephrin-B1-pTyr(324/329) showed differences concerning ephrin-B1 phosphorylation and its timing for mutant and wt protein. This may indicate that other Tyr-residues are more important for signalling. Alternatively, disrupted signalling through the Eph-B2-receptor contributes to the disease manifestation.

## P02.169

**EVC1 and EVC2 gene mutations associated to Ellis-van Creveld Syndrome**

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<sup>2</sup>Medical Genetics, Bambino Gesù Hospital, Rome, Italy, <sup>3</sup>Department of Experimental Medicine and Pathology, "La Sapienza" University of Rome, Rome, Italy. Ellis-van Creveld syndrome (EVC, MIM #225500) is a chondral and ectodermal dysplasia characterized by short ribs, polydactyly, growth retardation, and ectodermal and heart defects. This rare genetic disease is inherited as an autosomal recessive trait with variable expression. In general population the incidence is reported as one per 60000 live births. Mutations of EVC1 and EVC2 genes, located in a head to head configuration on chromosome 4p16, have been identified as causative. EVC1 gene has 21 coding exons spanning 120kb of genomic DNA and gives rise to a 992 amino acid protein. EVC2 gene has 22 coding exons spanning 150kb of genomic DNA and encodes a 1,308 amino acid protein. In this study we screened for mutations in both genes a panel of 33 EVC patients originated from different countries and collected relative clinical data. The analysis were conducted by DHPLC and direct sequencing of the PCR products resulting from amplification of all coding exons. We identified 22 mutations (16 never described in literature), 16 in EVC1 gene and 6 in EVC2. In EVC1 we identified 8 splicing, 5 frameshift, 2 missense mutations and a large deletion spanning almost all exon 13 (82419\_82561del). In EVC2 gene we described 3 frameshift, 1 splicing, 1 missense and 1 nonsense mutations. Our study expands the spectrum of mutations associated with EVC syndrome and will contribute to further understanding of the molecular basis underlying the phenotypic variability of the disease.

## P02.170

**Alpha thalassemia: sometimes a complex diagnosis**

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We report the case of a couple, coming from Philippines, referred to our Laboratory during the pregnancy for suspected alpha-thalassemia. We performed the routine analysis for the 7 most common muta-

tions, using gap-PCR for deletions and restriction analysis for point mutations. The male resulted heterozygous Alpha2IVS1-5nt, while the woman was negative. We checked the results using a RDB commercial kit, able to detect 22 alpha-globin mutations.

The female showed a double gene deletion(-SEA), not tested in the first approach, while the point mutation was not present in the male. Following direct sequencing we can detect a novel deletion of 24 bp adjacent to the 5nt-pathogenic deletion: this variation affected the restriction site used for the first diagnosis by RFLP. However, a bioinformatic analysis excluded a possible functional meaning. Since the female had performed an amniocentesis for advanced maternal age, we explored the presence of these mutations in foetal cells too. We found the-SEA, but not the 24nt-deletion. We confirmed by MLPA the prenatal diagnosis, but unexpectedly MLPA failed to amplify one probe: 8488-L8410, located between HBA1P and HBA2. Sequencing the corresponding region we identified a SNP in the father and in the foetus, responsible for the lack of amplification at MLPA.

Failing the genotype characterisation, the couple would be wrongly considered at risk for HBH in their children. This experience allowed us to conclude that molecular characterisation of globin genes, although widely and routinely diagnosed, must be performed by laboratories with consistent cultural background and with availability of a panel of articulated methods for assuring accurate testing.

#### P02.171

##### MLPA: A screening tool for molecular diagnosis of unknown alpha thalassemia deletions

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**Introduction:** Alpha thalassemia is the most common hemoglobin disorder in Iran. Most of known alpha thalassemia mutations include deletion of one or both alpha globin genes. Its importance is mainly due to its role in making a correct decision for performing prenatal diagnosis (PND) and differentiating unknown alpha thalassemia from normal HbA2 beta thalassemia. MLPA, a recently developed simple technique suitable for rapid quantitative analysis of deletions and duplications, was used to determine new deletion in cases suspected of alpha thalassemia.

**Material and Methods:** Couples referred to Kawsar Genomics Center for PND investigated for common deletional alpha-globin mutations and point mutation using multiplex Gap-PCR and direct sequencing respectively. Those remained unknown, further investigated for other deletions by MLPA methods which performed. After denaturation, hybridization and ligation, PCR-amplification was performed with the specific SALSA primers. Electrophoresis of PCR products performed using ABI-3130 genetic-analyzer.

**Result:** Thirty three suspected cases with low MCV, low MCH and normal HbA2 included in the study. No alpha globin gene mutation was detected in conventional investigation for common deletions and point mutations. In MLPA study of globin gene cluster a variety of diverse deletion patterns in probe set were seen in 23 (69.7%) of cases expanding from downstream of beta gene up to HS-40. Deletion lengths varied from at least 5.8 kb up to 67.2 kb.

**Conclusion:** This study, showed using MLPA can help us to increase accuracy of prenatal diagnosis for alpha thalassemia especially when we face with cases suspected to have large deletion.

#### P02.172

##### Transfusion dependent H disease: caused by compound heterozygote of two point mutations in alpha globin gene

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Thalassemia is the most prevalent genetic disorder. It can be of different severity characterized by an imbalance of  $\alpha$  and  $\beta$ -globins genes expression leading to anemia. H disease is the result of mutation in 3

alpha globins genes. However, it can also be result of coinheritance of 2 point mutations. Here we report a case of transfusion dependent H disease.

DNA was extracted from peripheral blood cells according to the protocols. Alpha and beta globins gene cluster was investigated using direct sequencing and real time PCR.

A couple with hematologic picture of alpha thalassemia (low MCV, MCH, and normal HbA2) were referred to our center (i.e. Father with MCV=73.6 Fl, MCH=21.6 pg, Hb=12.6 g/dl and HbA2=2.7 and the mother with MCV=73.5 Fl, MCH=22.2 pg, Hb=11g/dl and HbA2=2.2) They had an 8 years old child with more severe hematological presentation (i.e. MCV=62.7 Fl, MCH=16.1 pg, Hb=5.1 g/dl and HbA2=2.5) who received blood transfusion once a month since 7 years old.

Molecular study revealed no mutation in beta globins gene in the child or the parents. Direct sequencing of alpha globins genes showed 2 point mutations in alpha II gene of the affected child (i.e. poly A1: AATAAA>AATAAG and C59: GGC>CGC).

Poly A1 had been inherited from the father and C59 from his mother. This report indicates the importance of alpha globins gene point mutation and the possible need for prenatal diagnosis. There is a need for more reports on similar cases to provide a better genetic counseling for at risk couples.

#### P02.173

##### Low HbA2, beta thalassemia; not very prevalent but important

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**Introduction:** Beta thalassemia is one of the most prevalent monogenetic disorders in the word and also in Iran. Those who carry one mutation in beta globin gene usually present with hematologic picture of microcytic hypochromic anemia with high HbA2. Mutation in IVSII-nt1 in the beta globin gene is one of those, which usually presents with HbA2 higher than 3.5 g/dl.

**Material and methods:** Couples referred to us for prenatal diagnosis (PND) of thalassemia were investigated using ARMS PCR technique and direct sequencing.

**Results:** From those investigated for beta thalassemia mutation in the past 8 years, 23 cases (less than 2 %) of those with IVSII-nt1 mutation, had normal HbA2. From which, 9 cases (39.1%) showed high HbF ( $\geq 1.5$ ) (range: 1.6-11.5 g/dl) with hematologic picture of MCV: 51-70.4; MCH: 17.1-22.3; Hb: 9.9-14.6 and HbA2: 1.7-3.4. Cases with low HbF ( $< 1.5$ ) (range: 0.5-1.1 g/dl) didn't show any significance differences in hematologic picture with the previous group. (MCV: 59-78.6; MCH: 18-28; Hb: 10.2-15.5 and HbA2: 2-3.5).

**Conclusion:** IVSII-nt1 is one of most prevalent beta globin mutation in our country and as we observed, in near to 2% of the cases can present with normal HbA2. With considering high HbF, it can be diagnosed in only half of the cases. To be more accurate and not to miss others with normal HbA2 and HbF, we suggest in couples that one partner is carrier of thalassemia minor; beta globin gene mutation should be excluded in the other one even with normal HbA2.

#### P02.174

##### Preimplantation genetic diagnosis of beta-thalassemia by using fluorescence resonance energy transfer (FRET) hybridization probes and melting curve analysis: A case report

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**Background:** Preimplantation genetic diagnosis (PGD) is being employed increasingly, allowing transfer healthy embryos to the uterus. Validation of PCR-based assay is challenge because only one blastomere will be available, and the amount of DNA is insufficient for a given embryo.

**Material and Methods:** We developed a valid single-cell PCR protocol for PGD by using PCR followed by fluorescence resonance energy transfer (FRET) hybridization probes and melting curve analysis. We

optimized and clinically applied the protocol permitting molecular genetic analysis to amplify a specific region on the beta-globin gene for a couple, carriers of two mutations c.-78A>G and c.52A>T. Among a total of eight embryos were obtained after ovarian stimulation, a single blastomere per embryos were biopsied.

**Results:** The PCR fragments (364 bp) encompassing the both mutation sites were successfully amplified. A single base mismatch resulted in a melting temperature (Tm) shift of 8 °C for c.-78A>G mutation and 5 °C for c.52A>T mutation, allowing distinguish a wild type allele from the mutant allele. Genetic diagnosis showed that four embryos were unaffected embryos and two embryos were selected to transfer achieving pregnancy. Finally, a healthy boy was born.

**Conclusion:** Based on our results, this strategy can be successfully performed for PGD by using single-cell PCR. It is suitable as a noninvasive clinical tool for monogenic diseases.

#### P02.175

##### National campaign for the control of thalassemia in Iran

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Thalassemia is regarded a major health problem in many parts of the world. Population migration from thalassemia endemic regions to North and South America as well as Europe has made it a world problem.

In Iran National Thalassemia Screening Program for prevention of transfusion dependent thalassemia began in 1997. Premarital screening became compulsory and at risk couples are dealt with a comprehensive program. The program includes test confirmation, iron therapy, hematological consultation, genetic tests, prenatal diagnosis (PND) and legal therapeutic abortion of the affected fetuses. PND is covered by health insurance. National guidelines have been devised for every steps including for PND. Laboratories in the program are connected to each other as networks including prenatal diagnosis laboratories network. Their performances are monitored and regular inspections are carried out by members of Experts Committee. Every case of PND has to be reported to the Genetics Office, Ministry of Health. National Reference Laboratory for PND was created. In provinces with no medical genetics center, new centers have been created by tech-transfer using the experience and expertise from National Reference Laboratories. More than 10,000 prenatal diagnoses have been performed by these laboratories. In our center more than 3500 PNDs have been performed with only one misdiagnosis. Majority of referred cases were tested for beta-thalassemia. ARMS and RFLP techniques are used for most cases. In other cases direct DNA sequencing, Real-time, MLPA, SNPs and newly developed STR markers are used to come to a final conclusion.

#### P02.176

##### Developing a new STR marker to aid prenatal diagnosis of beta thalassemia

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Thalassemia is the most prevalent genetic disorder in Iran. Mutation in beta globin gene, in homozygote state, may causes severe clinical picture of thalassemia major. Here we report a case of combined alpha-beta mutation which couldn't be diagnosed with conventional molecular studies.

A couple was referred to us for prenatal diagnosis (PND). The couple's hematological parameters were: (husband: MCV: 67.9; MCH: 21.0; HbA2: 5.3%; wife: MCV: 72.9; MCH: 22.2; HbA2: 5.3%).

The mutation in the husband was found to be IVSII-nt1 in the beta globin gene. Our investigation on the wife's DNA using ARMS, MLPA, Real-time PCR techniques and DNA sequencing revealed no mutation. Repeated hematological analysis gave similar results (i.e. low MCV, MCH and high HbA2). We also investigated her alpha-globin gene for possible mutation using similar techniques as above. No mu-

tation was detected either. We then anticipated that the mutation causing imbalance in beta-globin synthesis may lie elsewhere. We tried to use known SNP or RFLP markers to use indirect methods for PND. No site was informative. We used a newly developed globin-gene linked STR markers to do so. Her father was carrier as well (i.e. MCV: 74.5; MCH: 18.8; A2: 4.6%). The primers for this STR are given below: F=T GCTAAGTAGATGCTGAGTGGC, R=GCCAATACTGCTGCATCATG. The sizes were: M:253/256; MM: 249/253; MP: 253/256. In this regard the mutant gene is linked with alleles 253. Use of STR markers are preferred compared with SNPs since STRs are usually multi allelic and there is more probability of being informative.

#### P02.177

##### Arrayed Primer Extension (APEX) and SNP Analysis for the Non-Invasive Prenatal Diagnosis (NIPD) of β-thalassaemia

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β-thalassaemia is the most common autosomal recessive single gene disorder in Cyprus. Prenatal diagnosis of β-thalassaemia is based on chorionic villus sampling that poses an abortion risk to the fetus. The discovery of cell-free fetal DNA in maternal plasma has opened up new possibilities for Non-Invasive Prenatal Diagnosis. The development of a NIPD assay for β-thalassaemia is based on the analysis of maternal plasma DNA for the detection of the paternally inherited fetal alleles using Single Nucleotide Polymorphisms (SNPs) and the Arrayed Primer Extension (APEX) assay.

A SNP genotyping analysis was performed on 75 random samples from the Greek-Cypriot population using 130 SNPs located across the β-globin cluster for the determination of heterozygosity. 48 SNPs with more than 10% heterozygosity were selected for the development of NIPD. Moreover, 55 families at risk for carrying a β-thalassaemic fetus were analyzed using 11 SNPs in order to determine which were informative for NIPD.

For the development of the APEX assay we designed a DNA microarray chip called "thalassochip" containing 60 β-thalassaemia mutations and 18 SNPs located across the β-globin locus. The sensitivity and specificity of the approach was determined. 24 maternal plasma samples were analyzed using the APEX assay for 2 SNPs. NIPD was successfully performed on 17 samples, 2 were misdiagnosed, whereas 5 analyses failed.

The APEX assay is a promising technique for NIPD and more tests with additional SNPs need to be performed in order to render the assay 100% reliable for NIPD.

#### P02.178

##### Simultaneous alpha and beta carriers may caused severe H-Disease

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Thalassemia is a major health issue in Iran. Premarital screening has been in effect since 1997. Couples with low MCV and/or MCH are detected and if needed are referred to one of several medical genetics laboratories throughout the country.

A couple was referred to our center for prenatal diagnosis of thalassemia. The male partner had low hematological data comparable with thalassemia (i.e. MCV: 63.5 fL, MCH: 19.8 Pg, Hb: 15 g/dL and HbA<sub>2</sub>: 2.5 g/dL). He was diagnosed to be carrier of alpha thalassemia with Med mutation (-Med/ αα). The female partner's hematological data was: MCV: 80 fL, MCH: 25.6 Pg, Hb: 14.2 g/dL, HbA<sub>2</sub>: 5.4 g/dL, HbF: 6.6 g/dL. At first glance she would be diagnosed as being a carrier of beta-thal. Molecular analysis showed she was both carrier of alpha and beta thalassemia. The high level of HbA<sub>2</sub> was indicative of beta-carrier and near normal MCV and MCH was indicative of being carrier of both beta and alpha-thal. We could detect 3.7 kb deletion in alpha globin gene. The risk of H-disease for the fetus is 25% though it would be mild. The parents should become aware of this fact. Another point is that since point mutation in alpha-genes can give similar hematological result and then in such case a transfusion dependent H-disease may

result. It can be suggested that when one couple carry a severe form of alpha-thal as cis the other partner should be checked for alpha-gene mutation even if he is carrier of beta-thal.

#### P02.179

#### Segmental duplications involving the $\alpha$ -globin gene cluster as a modulating factor in $\beta$ -thalassemia intermedia.

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Recently we have described two cases of heterozygosity for the common  $\beta^0$ -thalassemia mutation  $\beta39$  ( $C \rightarrow T$ ) presenting with a thalassemia intermedia phenotype (1). Multiplex Ligation dependent Probe Amplification (MLPA) analysis of the  $\alpha$ -globin gene cluster revealed two new rearrangements, consisting of a full duplication of the  $\alpha$ -globin genes locus including the upstream regulatory elements. Here, we present two other case; one, a family of mixed Sephardic Ashkenazi Jewish origin living in Canada, in which the propositus (a 2 yrs old girl) presented with a pronounced microcytic hypochromic anemia, a borderline HbA2 and heterozygous for a  $\beta$  polyA mutation. Only one parent showed mild microcytic hypochromic anemia due to heterozygosity for the  $\beta$ -mutation. The second case, a 6 yrs old girl of middle-eastern origin living in the U.K., has severe anemia and splenomegaly, while the mother from whom she has inherited the  $\beta^0$ -thalassemia mutation is clinically asymptomatic. MLPA analysis of the  $\alpha$ -globin gene cluster revealed two new rearrangements, consisting of a full duplication of the  $\alpha$ -globin gene cluster and the upstream regulatory elements. The first is a duplication of approximately 435 kb between position 75563-510533 from the telomere (UCSC Genome Browser, May 2004) and the second, approximately 107 kb between positions 90548-198161. We report the clinical and hematological data and the molecular characterization. We conclude that  $\alpha$ -globin gene duplication is more common than previously thought and should be investigated as a contributing cause in all unexplained unusually severe heterogeneous  $\beta$  thalassemia.

(1) Harteveld et al. Blood Cells Mol Dis. 2008 May-Jun;40(3):312-6.

#### P02.180

#### $\alpha$ -Thalassemia: report on the last three years of activity

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$\alpha$ -Thalassemia is the most common of the inherited hemoglobin synthesis disorders, which are the most common monogenic diseases. It is characterized by decreased or complete absence of  $\alpha$ -globin chain synthesis, caused by deletion of or mutation in the  $\alpha$ -globin genes. Clinically, 4 variants of the syndrome are recognizable, with increasing severity depending on how many normal  $\alpha$ -globin genes are present (3, 2, 1 or none). During the last three years of activity we have tested 109 individuals characterized by low MCV, normal or slightly reduced Hb levels and normal HbA<sub>2</sub>.

Thirty single gene deletions ( $\alpha$  3.7), twenty one double gene deletions ( $\text{--SEA}$ ,  $\text{--MED}$ ,  $\text{--FIL}$ ,  $\alpha$  20.5) and 12 point mutations (Hb Icaria, Torino, Constant Spring, Sun Praire,  $\alpha$ 2 init cd T>C, polyA) were analyzed by Reverse Dot Blot and direct sequencing, and  $\alpha$  thal mutations were identified in 106 cases. In 12 individuals none of these mutations was found.

The following genotypes were observed:  $\alpha$ 2 IVS 1-5 nt (12 subjects),  $\alpha\alpha$  (3), polyA/Hb Icaria (1),  $\alpha$  3.7--MED (1),  $\alpha$  3.7--SEA (1),  $\alpha$  3.7--FIL (1),  $\alpha$  3.7/ $\alpha$ 2 init cd T>C (4),  $\alpha$  3.7/ $\alpha$  4.2 (1),  $\alpha$  3.7/ $\alpha$  20.5 (4),  $\alpha$  3.7/Hb Hasharon (2),  $\alpha$  3.7/Hb Torino (1),  $\alpha$  3.7/ $\alpha$ 2 IVS 1-5 nt (2),  $\alpha$  20.5/ $\alpha$ 2 IVS 1-5 nt (1).

Our study shows that the  $\alpha$  3.7 single gene deletion is a very frequent cause of  $\alpha$ -thalassemia in Italy but other mutations ( $\text{--SEA}$ ,  $\text{--MED}$ ,  $\text{--FIL}$ ,  $\alpha$  20.5) also seem to have acceptable frequencies.

#### P02.181

#### New observations and it's consequences on genetic counselling: a case of $\beta$ -Thalassemia and Hereditary Hemochromatosis coexistence in homozygosity

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Genetic counselling is an integral and expanding part of medical practice, not only for those patients at risk, but also for patients with common disorders with genetic component. Furthermore, together with a fact of extreme velocity in application of new techniques and technologies in a field of genetic testing procedures, make that sometimes a role of genetic counsellor is completely forgotten.

Herein, we discussed some observations regarding double homozygosity for two well studied illnesses [ $\beta$ -thalassemia ( $\beta$ -thal) and hereditary hemochromatosis (HH)]. We reevaluate patients previously characterized for: HBB (Hemoglobin beta locus) and HFE (major gene associated with HH) respectively by our department. Our attention was directed to an 18-year-old patient, who was a double homozygote for  $\beta$ -thal [IVS1-110(G>A)/IVS1-110(G>A)] and HFE (H63D/H63D) who, at 16 years of age, had cardiomyopathy with severe hypogonadism. On the bases of results observed, it seems possible to suggest that the HFE gene homozygous H63D mutation, which is responsible for an adult onset of HFE, may be sufficient to provoke a juvenile hemochromatosis phenotype, when in combination with  $\beta$ -thal homozygosity. Even though, this observation open a possible "way" to speculate regarding synergic (pejorative) effects of these two genes, it seems extremely interesting to pay attention to possible cardiac problems, especially for young  $\beta$ -thal patients with high iron overload and HH homozygosity for mutation in the HFE gene. If confirmed, with further, larger studies, this observation could be interesting from both points of view i.e. clinician's and counselor's.

#### P02.182

#### Molecular diagnostics of thalassemia Intermedia in Iran

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To improve the differentiation of thalassemia intermedia from other hemoglobinopathies in Iran, four known genetic mechanisms\_XmnI Ggamma polymorphism, inheritance of mild and silent beta-thalassemia alleles, delta beta deletion, and coinheritance of alpha- and beta-thalassemia\_were investigated in 52 Iranian individuals suspected to have thalassemia intermedia based on clinical and hematological characteristics. Beta globin mutations were studied using a reverse-hybridization assay and sequencing of the total beta-globin gene. The XmnI Ggamma polymorphism, the Sicilian delta beta deletion, and four alpha-globin mutations (\_a3.7, \_a4.2, \_MED, aaa anti-3.7) were studied using PCR-based techniques. The inheritance of the XmnI Ggamma polymorphism with severe beta-thalassemia alleles in the homozygous or compound heterozygous state was the predominant mechanism observed in 27 individuals (55.3%). In five cases, this status overlapped with the \_a3.7=aa genotype. The second most frequent cause for thalassemia intermedia (14.8%) was the inheritance of mild beta-thalassemia alleles, including IVS-I-6 (T>C), \_88 (C>A), and +113 (A>G). In three subjects (4.3%) the Sicilian delta beta deletion was identified. HbS in association with beta-zero-thalassemia was found in three patients with thalassemia intermedia phenotype. In 11 cases (21.3%) no causative genetic alteration could be identified. Our results reflect the diversity underlying thalassemia intermedia, and the limitations of the applied clinical, hematological, and molecular approaches for correct diagnosis. Some of the unresolved cases will offer an opportunity to discover additional molecular mechanisms leading to thalassemia intermedia.

## P02.183

**Five novel *CDKL5* mutations in girls with severe mental retardation, early onset epilepsy, movement disorder and lack of expressive speech**

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Mutations in the X-linked cyclin-dependent kinase-like 5 (*CDKL5*) gene have been reported in the last years in nearly 50 patients as a cause for a neurodevelopmental disorder, characterized by drug resistant early-onset seizures, movement disorder, microcephaly and severe psychomotor impairment.

We screened the *CDKL5* gene for mutations in a cohort of 20 female and six male patients. Clinical information was available for 24 of them. All these patients showed epilepsy and moderate to severe mental retardation. 12 patients showed microcephaly, 13 hypotonia and two had dysmorphic features. Movement disorder (ataxia, athetotic movements) was present in ten patients. We detected five novel and one reported heterozygous mutations in the *CDKL5* gene in six female patients aged two to 16 years. The mutational spectrum consisted of three frame-shift mutations, leading to a premature stop codon, two missense and one nonsense mutation. One patient with a missense mutation has been already published (PMID 15499549).

Our patients with *CDKL5* mutations show a consistent phenotype including treatment resistant seizures starting in the first months of life after a normal newborn period. Additional features are hypotonia, severe psychomotor retardation, microcephaly, stereotype hand movements as well as ataxia and restlessness. Feeding difficulties, gastroesophageal reflux and scoliosis were present in some patients. All our patients with *CDKL5* mutations lack expressive speech.

The clinical findings in our patients with *CDKL5* mutations confirm that *CDKL5* deficiency represents a consistent phenotype. We discuss the clinical features with respect to the main differential diagnoses like Rett and Angelman syndromes.

## P02.184

***MECP2* de novo duplication in a girl with mild mental retardation and no obvious dysmorphic features**

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Mutations of *MECP2* are responsible for Rett Syndrome (RS), an X-linked neurodevelopmental disorder affecting mainly girls. The availability of *MECP2* testing has led to the identification of mutations in girls with atypical RS features and the recognition of milder forms. Furthermore, duplication of the entire gene has recently been described in boys presenting a different clinical picture, featuring severe mental retardation and recurrent infections.

We describe a girl with a heterozygous *MECP2* duplication. The patient, aged 19 years, has mild mental retardation, a kind, friendly but anxious character. She has neither dysmorphic features nor malformations. As a child she presented no particular health problems. Her motor development was slightly delayed with walking at 20 months, hypotonia and minor balance difficulties but otherwise within normal limits. Speech is fluid with good pronunciation but is simple and repetitive. Diagnosis was made after sequencing and MLPA analysis of *MECP2*. Parental analysis demonstrated that the duplication was *de novo*. CGH analysis defined a duplication of 0.129Mb (from position 152.930 Mb to 153.059Mb) long, including *MECP2* and part of *IRAK1* genes. X-inactivation is not skewed (in leukocyte DNA).

We conclude that it is highly likely that this duplication has phenotypic consequences.

These findings raise considerable issues concerning the diagnosis of girls with mild mental retardation and no dysmorphic signs and the genetic counselling for the parents.

## P02.185

**Congenital variant of Rett syndrome due to the *FOGX1* gene.**

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Rett syndrome is a severe neurodevelopmental disorder representing one of the most common genetic causes of mental retardation in girls. The classic form is caused by *MECP2* mutations. By candidate gene approach, we recently identified *FOGX1* as responsible for the congenital variant of Rett. *FOGX1* encodes a brain-specific transcriptional repressor, essential for early development of the telencephalon, that exhibits an expression pattern in the postnatal cortex partially overlapping with that of *MECP2*. Sixty *MECP2/CDKL5* mutation-negative European Rett patients (classical and variants), 43 patients with encephalopathy with early-onset seizures and 4 atypical Rett patients were analyzed for mutations in *FOGX1*. Mutations of *FOGX1* were identified in 4 patients, independently classified as congenital Rett variant from France, from Spain and from Latvia. Clinical data were compared with the two previously reported *FOGX1* mutated patients. In all cases there is an early onset of symptoms. In the perinatal period the girls are floppy, passive and easy to cry. Deceleration of head growth starts before the fourth month and leads to severe microcephaly. Motor development is severely impaired and the voluntary hand use absent. In contrast with classic Rett, patients have poor eye contact. Typical stereotypic hand movements with hand-washing and hand-mouthing activities were constant and present all time. Some patients present tongue movements. Jerky movements of upper limb were also present. Brain MRI shows corpus callosum hypoplasia in most cases, while epilepsy is a variable sign. Scoliosis is usually severe. Neurovegetative symptoms typical of Rett are frequently present.

## P02.186

**A 2,8 Mb 14q12 deletion in a boy with severe encephalopathy and Rett-like features**

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We report on a six-years- old boy with a *de novo* 2,8 Mb interstitial deletion of chromosome 14q12 identified by array-CGH. He presents a severe mental retardation, post-natal microcephaly, epilepsy, jerky movements of the upper limbs and minor dysmorphic features: large ears and a tented upper lip. *FOGX1* gene, which encodes a brain-specific transcriptional repressor essential for early development of the telencephalon, is the only gene entirely included in the deleted region. Alteration of *FOGX1* has been previously described in four girls with severe mental retardation, seizures and Rett-like features: jerky movements of the upper limbs, stereotypic activities, abnormal breathing patterns. Two girls presented a *de novo* 14q 12 deletion detected by array-CGH ( Bisgaard, 2005; Papa, 2008), the others had truncating mutation of *FOGX1* gene ( Ariani, 2008). These patients and our case report suggest that haploinsufficiency of *FOGX1* gene may cause a severe encephalopathy mimicking the congenital variant of Rett syndrome. Array -CGH should be performed in boys presenting with such clinical features and in girls without *MECP2* mutation. *FOGX1* gene analysis should also be discussed if array -CGH is normal.

## P02.187

**Genetic Heterogeneity in Rett syndrome: Molecular and Clinical Characterization of Females Heterozygous for Deleterious Mutations in CDKL5 and FOXG1**

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The *CDKL5* and *FOGX1* genes have been implicated in the molecular etiology of variant forms of Rett syndrome. We screened the *CDKL5* gene in a cohort of 167 patients with early-onset seizures and 10 girls with Aicardi syndrome. The screening was negative for all males as well as for females with Aicardi syndrome, excluding the *CDKL5* gene as a candidate for this neurodevelopmental disorder. We found 11 additional *de novo* mutations in *CDKL5* in female patients with a high mutation rate (28%) in females with early-onset seizures and infantile spasms. In addition, we screened the entire coding sequence of *FOGX1* in a cohort of 35 female patients with a classical or congenital form of RTT and 6 males with neonatal epileptic encephalopathies or RTT-like phenotypes. We found two female patients to be heterozygous for nonsense mutations resulting in truncated FoxG1 transcription factors. One patient is affected by a classic form of RTT, whereas the other patient presents with a congenital variant of RTT. These findings give additional support to the genetic heterogeneity in RTT, and help to delineate the clinical spectrum in the phenotypes associated with *FOGX1* and *CDKL5* mutated alleles.

## P02.188

**A novel p.Arg970X mutation in the last exon of the CDKL5 gene resulting in a female with Rett syndrome-like features and mild seizure disorder**

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Introduction: Mutations in the *CDKL5* (Cyclin-Dependent Kinase-like 5) gene in Xp22 are associated with therapy-resistant seizures and atypical Rett Syndrome (RS) features in early infancy along with severe mental retardation. RS is a serious neurodevelopmental disorder caused by mutations in the *MECP2* gene in Xq28. In its classic form RS mainly affects females who after a relatively normal development during the first six months of life develop a wide spectrum of symptoms. Several atypical forms of RS also exist, such as the Hanefeld variant with early-onset convulsions, suggesting the implication of other genes in the RS phenotype.

Material-Methods: We herein report a 14-year-old female with a RS-like clinical picture, severe mental retardation, and well-controlled seizures. Results: *MECP2* gene testing was negative, but subsequent sequencing of the *CDKL5* gene revealed a novel p.Arg970X (c. 2908 C>T) mutation in the last exon. The truncated protein only misses a small portion of the terminus, which might explain the less severe phenotype.

Conclusion: The study of the *CDKL5*-mutated variable phenotypes as the one presented here, may possibly contribute to the elucidation of the *CDKL5* and *MECP2*-associated overlapping molecular and clinical pathways.

## P02.189

**Epileptic encephalopathy in a girl with an interstitial deletion of Xp22 comprising promoter and exon 1 of the CDKL5 gene**

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We report a two-year-old girl with early onset seizures variant of Rett syndrome with a deletion at Xp22 detected by multiplex ligation-dependent probe amplification (MLPA) technique. This patient presented with tonic seizures at seven days of life. Subsequently, she developed infantile spasms at three months and finally refractory myoclonic epilepsy. She demonstrated severe encephalopathy with hypotonia, deceleration of head growth, with eye gaze but limited eye pursuit, no language, limited hand use, and intermittent hand stereotypies. This combination of clinical features, suggestive of early onset variant of Rett syndrome led us to screen the *CDKL5* gene. In a first step, screening of the whole coding sequence of the *CDKL5* gene revealed no point mutations. In a second step, we searched gross rearrangements by MLPA and identified a microdeletion affecting both the promoter and exon 1 in *CDKL5*. Subsequent analysis on a Nimblegen HD2 microarray confirmed a deletion of approximately 300 kb at Xp22, including the *BEND2*, *SCML2* and *CDKL5* genes. In conclusion, our report suggests that searching for large rearrangements in *CDKL5* should be considered in girls with early onset seizures and Rett-like features.

## P02.190

**MECP2 mutations in Bulgarian Rett syndrome patients**

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Rett syndrome (RTT) is a progressive neurodevelopmental disorder that occurs almost exclusively in females. It is characterized by arrested development between 6 and 18 months of age (with apparent normal development before that). The main clinical symptoms are: regression of acquired skills, loss of speech, stereotypical movements of the hands, microcephaly, seizures, and mental retardation. The typical form of RTT is caused by X-linked dominant mutation in the gene encoding methyl-CpG-binding protein-2 (MECP2).

In total 21 female patients clinically suspected to be affected by classical RTT were referred to our laboratory for genetic analysis of MECP2 gene. The MECP2 gene was screened for mutations by PCR/direct sequencing.

In one patient the karyotype analysis showed abnormal result - 46,XX,del(X)(p1.22).

Five of the affected girls (23.8%) were proved to have mutations in MECP2 gene: c.473C>T, p.Thr158Met; c.808C>T, p. Arg270X (detected in two unrelated patients); c.880C>T, p.Arg294X; c.1157\_1200del44, p.Leu386fs. The detected mutations were all *de novo*.

All of our genetically proved cases were very severely affected: total loss of speech is present in all cases, severe mental retardation, stereotypical hand movements are present, walking without help is impossible, seizures are invariably found in the presented girls.

Two of our patients were additionally tested for Angelman Syndrome (AS) by Methylation Sensitive PCR analysis; 5 girls were screened for mutations along the Cyclin-dependent kinase-like 5 (CDKL5) gene and 10 were analysed by MECP2 MLPA Kit for large deletions/duplications detection. These additional genetic tests revealed no genetic changes in analyzed patients.

## P02.191

**Mouse models of MeCP2 disorders share gene expression changes in the cerebellum and hypothalamus**

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A group of postnatal neurodevelopmental disorders collectively referred to as MeCP2 disorders are caused by aberrations in the gene encoding methyl-CpG-binding protein 2 (MECP2). Loss of MeCP2 function causes Rett syndrome (RTT), whereas increased copy number of the gene causes *MECP2* duplication or triplication syndromes.

MeCP2 acts as a transcriptional repressor, however the gene expression changes observed in the hypothalamus of MeCP2 disorder mouse models suggest that MeCP2 can also upregulate gene expression, given that the majority of genes are downregulated upon loss of MeCP2 and upregulated in its presence. To determine if this role of MeCP2 extends beyond the hypothalamus, we studied gene expression patterns in the cerebellum of *Mecp2*-null and *MECP2*-Tg mice, modeling RTT and *MECP2* duplication syndrome, respectively. We found that abnormal MeCP2 dosage causes alterations in the expression of hundreds of genes in the cerebellum. The majority of genes were upregulated in *MECP2*-Tg mice and downregulated in *Mecp2*-null mice, consistent with a role for MeCP2 as a modulator that can both increase and decrease gene expression. Many of the genes altered in the cerebellum were similarly altered in the hypothalamus. Our data suggest that either gain or loss of MeCP2 results in gene expression changes in multiple brain regions and that some of these changes are global. Further delineation of the expression pattern of MeCP2 target genes throughout the brain might identify subsets of genes that are more amenable to manipulation, and can thus be used to modulate some of the disease phenotypes.

#### P02.192

#### Molecular karyotyping reveals *CDKL5* Deletions as frequent cause of mental retardation of unknown origin

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In order to further elucidate the causes of mental retardation we performed a genome-wide copy number variant (CNV) survey in 160 patients with mental retardation of unknown origin using the Affymetrix 6.0 SNP platform.

Surprisingly, two of the 160 patients (1.3 %) showed microdeletions involving the *CDKL5* gene. Mutations in this gene were only recently identified as causative for an atypical variant of Rett-syndrome characterized by intractable early-onset seizures often accompanied by Rett-like features.

Patient 1 is a 12 months old girl, born after uncomplicated pregnancy, with secondary microcephaly, severe mental retardation, severe hypotonia and drug resistant seizures. Karyotyping, testing for Angelman syndrome and *MECP2*-analysis showed normal results. By array analysis a 230kb deletion on Chromosome Xp22.13, including parts of the *CXorf20* gene, the complete *SCML2* gene and exon 1 of the *CDKL5* gene was detected.

Patient 2, a 7 years old girl was born after an uncomplicated pregnancy and is severely mentally retarded. She had convulsions in her first year of life and shows stereotypical movements of her hands. Her speech is nasal and limited to a few words. *MECP2* and *TCF4* testing was normal. In this patient we detected a 157 kb deletion on the X-chromosome, including a large part of the *CDKL5* gene, furthermore the *RS1* gene and parts of the *PPEF1* gene.

Our findings demonstrate for the first time, that *CDKL5* microdeletions are a major pathomechanism in female patients with severe mental retardation and seizures and seems to constitute a relatively frequent cause of mental retardation.

#### P02.193

#### Two novel cases of congenital variant of Rett syndrome related to mutations in the *FOXP1* gene

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The forkhead box G1 (FoxG1) is a transcription factor that is critical for forebrain development where it promotes progenitor proliferation and suppresses premature neurogenesis. Recently, the *FOXP1* gene was implicated in the molecular aetiology of the congenital variant of Rett syndrome. So far, seven mutations have been reported. We screened

the *FOXP1* gene in a cohort of 206 *MECP2/CDKL5* mutation negative patients with severe encephalopathy and microcephaly (136 females and 70 males). The screening was negative in all males. Two *de novo* mutations (c.1248C>G, p.Y416X and c.460dupG) were identified in two girls. Both patients showed neurological symptoms from the neonatal period with poor reactivity, hypotony, and severe microcephaly. During the first year of life, both weakly progressed and presented feeding problems. At 5 years, girls showed severe neurological impairment with gross hypotonia, no language, convergent strabismus, and no voluntary hand use. Instead, both presented the combination of jerky movements, hand-mouthing and hand-washing activities. Although our patients demonstrate severe encephalopathy compatible with the congenital variant, several features previously highlighted in *FOXP1* mutations patients were not observed: absent eye contact, inconsolable crying during perinatal period, and thin corpus callosum. Other signs were not systematically observed: protruding tongue, scoliosis, and epilepsy. Although the overall frequency of mutations in *FOXP1* in females with severe mental retardation and microcephaly appears to be low (1.5%), our findings suggest the requirement to investigate both point mutations and probably gene dosage in the *FOXP1* gene in patients with severe encephalopathy with microcephaly, and some Rett-like features.

#### P02.194

#### DNA Resequencing and Variant Identification Using a Non-syndromic X-linked Mental Retardation (MRX) Panel

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The prevalence of X-linked mental retardation (XLMR) is estimated to afflict ~1/1000 males. To date, there have been ~90 X-linked genes implicated in causing XLMR with the majority of these genes being associated with syndromal MR (MRXS). A smaller set of genes on the X chromosome have been associated with nonsyndromal MR (MRX) where the only discernible feature is mental retardation. Significant overlap between these two sets of genes indicates that syndromal and nonsyndromal can both be caused by alterations in many of the XLMR genes. From a clinical perspective, proper diagnosis of males with nonsyndromal XLMR is more straightforward when a positive family history exists. Unfortunately, many undiagnosed males with MRX exist, and the absence of an X-linked pedigree makes the identification of their underlying etiology much more difficult. Indicative of this difficulty is that MRX accounts for roughly 2/3 of the total number of XLMR cases. To test for MRX, the Molecular Diagnostic Laboratory at the Greenwood Genetic Center has designed a resequencing panel consisting of 95 amplicons encoding the exons and intron junctions of 9 X-linked genes. To demonstrate the advantages of a new capillary electrophoresis (CE) instrument, 19 blinded probands suspected of having MRX were directly sequenced using the Greenwood MRX resequencing panel. Variant detection in the 9 genes across the 19 samples will be discussed. The MRX Resequencing Panel coupled to the Fast Resequencing Workflow highlights the advantages of using CE for DNA resequencing and variant identification across a large number of samples and genes.

#### P02.195

#### Developmental delay and a distinctive facial appearance in two families with *Xq25* duplications

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We have previously reported a duplication (1.2 Mb) at Xq25 using whole-genome array Comparative Genomic Hybridization in a 20-year-old man with syndromic mental retardation (MR) (Jacquemont et al. 2006).

This duplication contains four known genes, one of which is GRIA3 (Glutamate Receptor, Ionotropic, AMPA subunit 3). Mutations, deletion and partial duplication of the GRIA3 gene have been reported in males with non-syndromic MR (Wu et al., 2007; Chiyonobu et al., 2007).

Our goal was to look for other patients with duplication of GRIA3 by performing Multiplex Ligation-dependent Probe Amplification (MLPA). We selected about twenty patients with MR based either on their facial features or on their behavioral disorders.

We found a duplication of GRIA3 resulting from a 2.8 Mb duplication at Xq25 in a 4-year-old boy who had a similar facial appearance. These two patients presented a common clinical phenotype: psychomotor delay, hypotonia, mild mental retardation, pervasive developmental disorder and non specific cerebral MRI abnormalities associated with characteristic facial features including malar flatness, lower palpebral eversion, thick lips and hypotonic facies. In both cases, the duplication was maternally inherited. The mothers presented an X inactivation bias and were unaffected.

We suggest that the Xq25 region duplication may result in a clinically recognizable condition. In particular, facial dysmorphism could help to diagnose this microduplication in males with X-linked MR.

#### P02.196

#### A duplication encompassing the *SMS* gene involved in a X-linked mental retardation different from Snyder-Robinson syndrome

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Duplications on the X chromosome have been rarely reported in males with mental retardation and dysmorphism. In most of cases they are inherited from female carrier phenotypically normal. We describe the molecular characterization of a maternal interstitial dup(X)(p22.11p22.12) encompassing the spermine synthase (*SMS*) gene in two brothers with mental retardation.

Clinically the two brothers demonstrated mild mental retardation, epicanthus, short up-slanting palpebral fissures, short extremities and hollow feet. The first one presented a strabismus and cryptorchid testes, and the second a congenital club foot. Short hands and feet were also observed in the mentally normal mother.

An Integragen BAC-array showed a gain for CTD-2033A1 clone, not confirmed by FISH analysis. Agilent 44K oligo-array confirmed the gain and precise the size (460 Kb). Quantitative real-time PCR confirmed the duplication in both brothers and showed the duplication was inherited from their mother. The genes included in the duplication were *SMS* and *PHEX*.

We discuss the implication of *SMS* gene in the phenotype of our patients. *SMS* gene mutations were shown to be involved in the X-linked recessive mental retardation Snyder-Robinson syndrome (OMIM 309583). This syndrome is characterized by a marfanoid habitus with long thin hands and feet. We suggest that the *SMS* gene could be involved in another X-linked mental retardation than Snyder-Robinson syndrome. Skeletal abnormalities in our patients (short hands and feet) are the opposite of what is described in loss of function of *SMS* gene. Finally, this report contributes to the clinical and genetic delineations of the *SMS* gene defects.

#### P02.197

#### The role of miRNA-105 in the development of nonsyndromic mental retardation

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MicroRNAs are noncoding RNAs that regulate many cellular functions including cell proliferation, differentiation and apoptosis. They attenuate gene expression by pairing with the 3' UTR of target transcripts inducing RNA cleavage or translational inhibition. These small regulatory molecules are central to various physiologic processes and their disruption is associated with human diseases, particularly mental retardation.

The purpose of this study was to investigate miRNAs-105 role in the development of nonsyndromic mental retardation (MR). We analyzed rs10238918 SNP located in a miRNA-105 binding site of 3'UTR of NeuroD6 gene in nonsyndromic MR patients and control group. Neu-

roD6 is a member of the NeuroD family of basic helix-loop-helix (bHLH) transcription factors. It activates E box-dependent transcription in collaboration with TCF3/E47 and is a trans-acting factor involved in the development and maintenance of the mammalian nervous system.

The studied groups included 144 male patients with nonsyndromic MR (ICD-10) divided into two groups: severe and mild MR and 120 healthy controls. Genomic DNA was extracted from peripheral blood leukocytes by standard phenol/chloroform method. Genotyping was performed by the PCR-RFLP technique.

Significant differences in G allele frequency have been found between patients with severe form MR and mild form (OR=2.4; CI95% = 1.1-5.4), ( $\chi^2=4.4$ ;  $p=0.03$ ;  $df=1$ ). The frequency of G allele in group of patients with severe MR was 32.1%, which is higher than in mild MR (16.6%). One of explanations of these differences could be that G\* allele is involved in formation of the binding site to miRNA-105 thus probably suppressing the expression of NeuroD6 gene.

#### P02.198

#### Molecular characterization of the promoters of the X-linked mental retardation gene *JARID1C* and additional members of the *JARID1* gene family

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X-linked mental retardation (XLMR) is genetically heterogeneous disorder affecting approximately 2 in 1000 males. Causative mutations have been found in over 100 different genes, but a significant proportion of the cases are still without molecular diagnosis. The majority of mutations were so far detected in the protein coding regions of X-chromosomal genes. However, sequence changes in regulatory regions may result in similarly detrimental effects by influencing the transcription efficiency of the respective genes.

Therefore one can assume that part of the unsolved cases of XLMR may be due to promoter mutations. Thus, it is necessary to identify functional promoter elements in known XLMR-genes in order to determine where sequence changes can have functional consequences. Furthermore, the identification of factors involved in the transcription of these genes is also a way to find additional genes with a putative role in MR. Therefore we investigated the promoter region of one of the more frequently mutated XLMR-genes, *JARID1C*, for functionally relevant sequences.

As *JARID1C* encodes a transcription factor, mutations in target promoters might also cause MR. Thus, having evidence of regulatory interplay between *JARID1C* and its homolog *JARID1B*, we included *JARID1B* as well as the other *JARID1* family members (*JARID1A* and *JARID1D*) in our study. Using a dual-luciferase reporter assay to measure the activity of different parts of the 5'-flanking regions of these genes in HEK and SH-SY-5Y cells, we defined regions that are important for transcriptional activity in all four *JARID1* genes.

#### P02.199

#### Towards understanding the pathogenetic mechanism of *PQBP1* mutations in X-linked mental retardation

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We have found that mutations in the polyglutamine binding protein 1 (*PQBP1*) gene cause X-linked mental retardation. Identical and similar mutations result in high clinical variability, ranging from moderate mental retardation to much more severe forms, including microcephaly, short stature and spasticity. More recently we have begun to unravel the pathomechanism of this disease and have found that *PQBP1* mutant transcripts with a premature stop codon are partially degraded by nonsense mediated mRNA decay (NMD) and that *PQBP1* mutations cause nonsense-associated altered splicing (NAS). Interestingly, some of the mutations resulted in an upregulation of naturally existing *PQBP1* protein isoforms. Additional studies demonstrated that the *PQBP1* protein is part of a large multiprotein complex containing RNA-binding proteins that are established components of RNA granules and play distinct roles in post-transcriptional RNA regulation and metabolism. Interestingly, we have found that *PQBP1* is present in dendritic shafts of primary cortical neurons in discrete granular structures and co-localises with newly found interacting proteins in these granules. Remarkably, a fraction of the *PQBP1*-containing granules co-localised with the fragile X-mental retardation protein. Taken together, our find-

ings strongly suggest that PQBP1 plays a hitherto unknown role in post-transcriptional RNA regulation and metabolism. Disturbance of one or several of these processes may contribute to the clinical phenotype in patients with a PQBP1 mutation.

#### P02.200

##### A recurrent copy number gain at Xq28 in four families with mental retardation reveals a dosage-dependent severity of the phenotype and suggests a novel recombination mechanism

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In a study to elucidate the genetic defects in patients with X-linked mental retardation (XLMR) we performed X chromosome-specific BAC-array-CGH and identified a 0.3 Mb inherited apparent recurrent copy number gain at Xq28 in affected males of four unrelated XLMR families. All aberrations segregate with the disease and the carrier mothers show nonrandom X-inactivation. Interestingly, this region was duplicated in two families with nonsyndromic mild to moderate MR, triplicated in a third family with more severe characteristics, while in a fourth family with severe syndromic MR, this region was present in four copies. The aberrant region is located at 153.21 Mb to 153.53 Mb and harbors 18 genes of which three are highly expressed in brain (*RPL10*, *ATP6AP1*, *GD1*). Expression analysis revealed copy number-dependent increased mRNA levels in affected patients compared to controls, which correlates with the severity of clinical features. Our data strongly suggest that an increased expression of genes within this region results in impaired cognition in a dosage-dependent manner. Breakpoint analysis revealed recombination sites within two adjacent sets of low copy repeats which implies a yet unknown recombination mechanism. Finally, in patients with *MECP2* duplication the region described in this study is often completely or partially involved, but the contribution of their increased mRNA expression to the severe phenotype is probably masked by the strong effect of increased *MeCP2* levels. Our data thus demonstrate that a copy number gain of individual genes present in a contiguous genomic aberration can on itself result in a clinical phenotype too.

#### P02.201

##### Transcriptional behaviour of *SLC6A8* gene mutant alleles in creatine transport deficient patients

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In mammals, creatine (Cr) is taken from diet or can be synthesized endogenously. Creatine uptake by cells with high energy demands depends on a specific creatine transporter, CT1 encoded by *SLC6A8* gene. Although there are about 20 pathogenic variations described for the *SLC6A8* gene, their transcriptional profile has been poorly studied. Here, we focus our interest in characterizing at mRNA level three in-frame mutations p.Ala404Pro, p.Phe360del and p.Phe480del, identified in three Spanish CT1 patients with a clinical presentation of the disease ranging from less to more severe. We performed both RT-PCR analysis to characterize possible exon skipping events and quantification of *SLC6A8* mRNA levels using qRT-PCR. The cDNA behaviour did not show any aberrant splicing, failing to explain their different clinical phenotype. However, a different expression profile of the *SLC6A8* gene in the three CT1 patient's fibroblasts was observed. So, alleles causing a phenylalanine deletion showed a significant reduction in the *SLC6A8* mRNA levels, when compared to that measured for p.A404P allele or controls. To analyse if these changes could trigger mRNA degradation, patient's fibroblast were treated with different inhibitors of both protein synthesis and non mediated decay process

(NMD) such as emetine and puromicine. An 8 and 2 fold recoveries of *SLC6A8* levels were obtained for p.Phe480del and p.Phe360del respectively, when compares to corresponding controls. We hypothesised, that a reduced expression of *SLC6A8* gene, probably related to mRNA instability, could be relevant to explain the more severe presentation of the disease in these two patients.

#### P02.202

##### Autosomal dominant hereditary spastic paraparesis with dystonia in two Russian families

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Dystonia (DYS) is a very rare feature in 'complicated' hereditary spastic paraparesias (HSP+). In 2008, Gilbert et al described a large family with autosomal dominant (AD) HSP+DYS and mapped the locus in 2q24-q31. We diagnosed AD HSP+DYS in two families. Family 1 numbered 4 patients, a 31-year-old proband, her 40-year-old brother, their father, and her 2.5 year-old son. In the proband, severe spastic paraparesia (SP) started in childhood; DYS presented in 11 yrs as writer's cramp and then as torticollis, in 27 yrs DYS in hands started; yet, SP prevailed. Her son had severe SP since infancy looking like spastic cerebral palsy; followed up to 8 yrs he was non-ambulatory, with normal mental development and no DYS. In the not-examined brother, SP presented since youth as 'shuffling' gait; DYS started at 28 yrs as torticollis and progressed to a severe generalized form resistant to treatment and prevailing over SP. The father had 'shuffling' gait till 50 yrs when the gait deteriorated rapidly; dementia and psychosis also developed; he died in 54 yrs non-ambulatory and mentally disabled. In Family 2, 24-year-old male proband was examined. SP and DYS started in 7-8 yrs and progressed with generalized DOPA-resistant DYS prevailing over SP. Proband's father died in 58 yrs with no evident symptoms; deceased paternal grandmother and her sister were said to have adult-onset generalized hyperkinesia. Intrafamilial variability and anticipation are seen in both families. Family 2 suggests incomplete penetrance. Origin of dementia in the father in Family 1 is questionable.

#### P02.203

##### Atypical clinical presentation of leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL)

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LBSL, MIM 611105, is a "new" autosomal recessive disease with distinctive MRI and spectroscopy features. The causative gene *DARS2* codes mitochondrial aspartyl-tRNA synthetase. Along with some other recently described leukoencephalopathies, LBSL proved to be a not infrequent disorder. In the Department of metabolic disorders of Medical Genetics Research Center, 35 unrelated LBSL cases were verified by DNA analysis since 2007. Typical features are childhood onset, slowly progressive cerebellar ataxia, spasticity, and dorsal column dysfunction. We present a 12-year-old female patient with atypical feature of paroxysmal dyskinésias. They started in 1.5 yrs during a common febrile infection and reappeared, produced by motion, as involuntary body turn to the right with putting right leg aside and crying. Later on, paroxysms became persistent and of different appearance. By now, they occur many times a day, mostly in the beginning of motion after rest, and look like abrupt quick legs flexion with sitting on the ground for few instants, or just like legs flexion if the girl is in a sitting position. Other signs are moderate spasticity and ataxia; mental development is normal. Epilepsy, electrolyte disbalance, myotonia, and psychogenic disorder were excluded. LBSL was diagnosed in 12 yrs when MRI was recognized as characteristic for the disease. Compound heterozygosity for common *DARS2* mutations, c.228-20\_21delTTinsC and c.455G-T, was found. To our knowledge, paroxysmal dyskinésias in LBSL were not described previously; their persistence through many years is also remarkable. The dyskinésias may be of brain stem origin, like those in multiple sclerosis or brain stem stroke.

### P03. Cytogenetics

#### Recurrent spontaneous abortions due to a homologous Robertsonian translocation (13q13q)

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Balanced Robertsonian translocations are relatively common in different populations, occurring at a frequency of about 1 in 1100 livebirths. The proportion of these translocations that involve homologous chromosomes appears to be extremely small. Robertsonian translocation between two chromosomes 13 is a rare event, and should not manifest any obvious phenotypic effects. Theoretically, carriers of homologous Robertsonian translocations are unable to produce normal children since all their gametes should be either disomic or nullisomic for the chromosome involved in the translocation. Our case was a 31 year old woman who presented with three spontaneous abortions (at 12, 9 and 8 weeks, respectively). Her hysterosalpingography was normal. Her laboratory tests including FSH, LH, Progesterone, Prolactin, Toxoplasmosis, Listeriosis and TORCH were normal. She had three brothers and three sisters, all healthy and married, with a few normal children. Chromosome analysis was performed on cultured cells according to standard methods from the patient's peripheral blood samples. All analyzed mitoses had 45 chromosomes with a derivative chromosome consisting of the long arms of the two chromosomes 13. The resulting net imbalance was loss of the short arms of both chromosomes 13. Her karyotype showed: 45,XX, der(13;13)(q10;q10). Cytogenetic studies of her parents were normal.

#### P03.002

##### The 1q-syndrome: A case report and literature review

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Deletions of the long arm of chromosome 1 have been reported in numerous patients, with breakpoints occurring along the entire length of the q arm, causing clinical differences. Specific phenotypic findings, resembling a distinct syndrome, are present in cases with terminal deletions at 1q42 and 1q43. Monosomy 1q42-qter seems to be associated with characteristic manifestations. On these basis, molecular techniques are important in order to correctly define the deleted segment, and establish accurate genotype - phenotype correlations.

We provide a clinical description of a girl with de novo terminal 1q43-qter deletion.

The proband has been recognized as an infant with intrauterine growth retardation and generalized muscular hypotonia, microcephaly, epicanthal folds, telecanthus, enophthalmus, short eye openings with lateral blepharophimosis, small, broad and flat nose, low-set ears, high-arched palate and short neck. Valgus deformity of feet was present with short right Achilles tendon.

Magnetic resonance of the brain revealed agenesis of the corpus callosum, ventriculomegaly and enophthalmus. She has seizures.

Cytogenetic analysis of peripheral blood showed a de novo terminal chromosome 1 long arm deletion, confirmed with Subtelomeric FISH (Multiprobe-T-System, CytoCell). To define the extent of the deletion and the breakpoint location we performed 244k array-CGH (Agilent Technologies, USA) demonstrated a loss of 9.4 Mb genomic material and the breakpoint 1q43 (at 237.6 Mb).

The patient's features are compared with those of other patients with similar deletions, and variable phenotypic findings due to different deleted chromosomal segments are discussed.

#### P03.003

##### Partial monosomy of distal 2q and partial trisomy of distal 2p in an adult mentally retarded patient, derived from a paternal inversion

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We describe a 24 year old mentally retarded and dysmorphic Iranian male patient with partial monosomy for distal 2q and partial trisomy for

distal 2p. The patient's karyotype is 46,XY,rec(2)dup(2p)inv(2)(p25.1 q37.3)pat. Chromosome analysis in the father showed a pericentric inversion of chromosome 2, described as 46,XY,inv(2)(p25.1q37.3). The proband's karyotype is a recombinant product of the paternal inversion.

Parents are consanguineous, with one normal daughter and the affected son. Both father and grandmother are deaf. The patient has moderate mental retardation with developmental delay. The clinical features include prominent arachnodactyly in feet and slim lower limbs, speech delay, poor coordination, prominent supraorbital ridge, contracture deformity of PIP, hands ulnar deviation, mild genuvalgus, mild facies asymmetry and retrognathia, thick and malformed auricles, refractive errors (myopia and astigmatism), prominent nose, with lipoma on the face and multiple large acne and scars. Most of the recorded clinical features include the reported features of partial trisomy of distal part of the short arm of chromosome 2. However, additional clinical features which are not characteristic of the observed chromosome imbalances are present. Complementary work for further characterization of the deleted and duplicated chromosome segments is underway.

#### P03.004

##### Nonmalignant aneuploidization in the human brain: chromosome instability can mediate neurodegeneration in Alzheimer's disease and ataxia-telangiectasia

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Chromosome instability (CIN) manifesting as aneuploidy is usually associated with tumorigenesis. However, recent studies have shown that phenomena related to CIN (cell cycle errors, aneuploidy) are observed in the diseased (nonmalignant) brain tissues. The latter phenomenon was recently observed in neurodegenerative disorders. To test a possible link between CIN and neurodegeneration, we have analyzed interphase chromosomes in the Alzheimer's disease (AD) and ataxia-telangiectasia (AT) brain. By means of interphase chromosome-specific multicolor banding (ICS-MCB) and interphase FISH, we monitored chromosome complement in about 500,000 neural cells derived from the cerebral cortex of AD, AT and normal human brain (control samples). We have established the mean rate of stochastic aneuploidy per "mean" human chromosome as 0.5% in controls (the normal human brain, 95%CI 0.2-0.7%; SD 0.2%). The AT brain demonstrated a dramatic 2-to-5 fold increase of stochastic aneuploidy randomly affecting different chromosomes (mean 2.1%; 95%CI - 1.5-2.6%; SD 0.8%). The overall proportion of aneuploid cells in the brain of AT individuals was estimated at 20-50%. The level of stochastic aneuploidy in the AD brain was slightly increased compared with controls. However, a dramatic 10-fold increase of cells with unstable chromosome complement affected by chromosome 21 hypoploidy and hyperploidy was detected in the AD brain (6-15% versus 0.8-1.8% in control). Thus, CIN manifested as aneuploidy does present in the AD and AT brain. Our data indicate that CIN mediated by neural aneuploidization does not lead to tumorigenesis but potentially mediate neurodegeneration in these devastating brain diseases. Supported by Philip Morris USA.

#### P03.005

##### Evaluation of Amenorrhea: Cytogenetic investigation in a Tunisian Cohort

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Chromosomal abnormalities described in primary and secondary amenorrhea range from X chromosome abnormalities such as Turner syndrome to assorted deletions and translocations to mutations in specific genes.

Sixty five women were referred to our Cytogenetic laboratory for chromosomal exploration because of primary or secondary amenorrhea.

Metaphase chromosomes were prepared using standard cytogenetic methods. Chromosomes were analysed by using RGH banding and karyotypes were interpreted according to ISCN 1995 nomenclature. The mean patient age was 28 years.

An overall frequency of 16,94 % chromosomal abnormalities was detected for our 65 patients. Sex chromosome abnormalities were predominant (90% of cases).

Among primary amenorrhea women, 16 had a 46,XX karyotype and 5 (26.3%) had abnormal karyotype: 45,X /46,XX , 46,X idic(X)(q11), 46,X,del(X)(q 21), 46,XY and 45,X /46,XY.

Karyotypes of patients with secondary amenorrhea were abnormal in 5 cases (12,5%) including four X chromosome mosaicism and one autosomal translocation: 45, X /46,XX , 46,XX /47,XXX, 45X/47,XXX and 46,XX, t(12 ;19) (q13 ;q13).

The present study has emphasized that karyotyping is one of the fundamental investigations in the evaluation of amenorrhea. Cytogenetic abnormalities seem to be more frequent in primary than secondary amenorrhea and mainly involve the X chromosome. Women with different cytogenetic types of X chromosome abnormalities should be diagnosed at young age and benefit from a carefully followed throughout life.

Genetic counseling is very hard for these patients but mutational analysis of candidate genes in 46,XX idiopathic amenorrhea is needed to determine which genes contribute to the cause of this disorder.

#### P03.006

#### Molecular neurocytogenetic survey of variations in chromosome numbers and arrangement in the schizophrenia brain

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It has been proposed that alteration of genome organization and behavior in neuronal cells could contribute to schizophrenia pathogenesis. However, related studies rarely address genomic variations at subchromosomal and chromosomal levels in the diseased brain. We have addressed variation in chromosome numbers and somatic chromosome pairing in neural cells of the schizophrenia brain by interphase FISH. We demonstrate that the schizophrenia brain exhibits increased rates of stochastic aneuploidy affecting different chromosomes with the presence of low-level mosaicism involving chromosomes 1, 18, 21 and X. Variation in chromosome number may destabilize genome in affected neuronal cells and play a definite role in schizophrenia pathogenesis. The study of interphase chromosome organization has showed the schizophrenic brain to differ in the rate of chromosomal associations by both heterochromatic and euchromatic regions. The frequency of somatically paired heterochromatic regions was 1.5-3 times higher for chromosomes 1, 9, 16 and 18 and for euchromatic regions of chromosomes 1 and 18, while pairing of chromosomes 15 and 17 has occurred more frequently in the non-diseased brain. Since nuclear organization defines proper functioning of a cell, the present findings propose for the first time alterations of chromosome organization in the brain as a possible epigenetic mechanism involved in the pathogenesis of schizophrenia. In summary, we can conclude that the schizophrenia brain is hallmark by specific behavior of neuronal genome which probably defines the cellular pathobiology of this common psychiatric disease. Supported partially by Philip Morris USA.

#### P03.007

#### Comparative analysis of numerical and structural chromosome aberrations in peripheral blood lymphocytes from workers upon exposure to plutonium-239

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Aneugenic mechanisms of activity for ionizing radiation have been debated for some time while its genotoxic and clastogenic action is well known. Six human chromosomes were investigated in pairs (2 and 8, 7 and 12, X and Y) for the purpose of comparative estimation of non-disjunction and lagging frequency in the binucleated human

lymphocytes from workers of nuclear-chemical industry and clinically healthy men of the same age using dual-color fluorescent in situ hybridization. In addition spectrum and level of structural chromosome aberration were estimated by G-banding technique. Statistically significant increasing of non-disjunction frequency has been found for all investigated autosomes in the cells of exposed individuals in comparison with controls. There were no differences between groups in the frequencies of gonosome non-disjunction. Chromosomal loss, which was detected as centromeric positive micronuclei, demonstrated no differences also. Analysis of the G-banded metaphase spreads has indicated that the main distinction between exposed and unexposed individuals was composed of the chromosomal aberrations such as deletions and translocations, which failed to be found using conventional solid-stained analysis. Thereby the internal exposure high-LET alpha-particle irradiation demonstrates aneugenic activity along with clastogenic effect, that can be result both alteration metaphase spindle and abnormal segregation of the rearranged chromosomes. So, it is advisable in the course of genotoxic investigations to use the analysis of aneugenic effects in addition to standard approaches. Using binucleated human lymphocytes coupled with FISH for non-disjunction assessment proved to be the most sensitive technique for detection of aneuploidy.

#### P03.008

#### Characterization by FISH and array-CGH of 26 apparently balanced chromosomal rearrangements associated with an abnormal phenotype

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Apparently balanced chromosomal anomalies affect around 0.5% of individuals. Regarding *de novo* reciprocal translocations, inversions and complex chromosomal rearrangements (CCR), mental retardation and/or congenital malformations are more frequent compared to the general population. We report 26 patients presenting abnormal phenotypes and chromosomal rearrangements considered balanced by conventional cytogenetics (20 reciprocal translocations of which one X-autosome, 5 inversions and 1 CCR), *de novo* (n=18) or inherited (n=8). All were analysed by oligonucleotide array-CGH: 8 turned out to be unbalanced, representing 25% of inherited cases, and 35% of *de novo* cases. All cryptic imbalances were deletions, ranging from 1 to 15 Mb, of which 2 were localized at distance from the breakpoints. For the remaining 18 patients, no quantitative anomaly was detected at 150 kb resolution. Few of them were selected to characterize precisely the breakpoints by FISH. Gene breakage was identified in two of them. In one case, a new gene implicated in facial clefts namely *FAF1* was identified. In the other case, disruption of *DMD* gene was revealed in a girl presenting a progressive dystrophinopathy. According to previous studies, although these are preliminary results, two major pathogenic mechanisms are underlined in this context: cryptic deletions and gene disruption. This suggests that array-CGH must be performed systematically when apparently balanced chromosomal rearrangement is associated with an abnormal phenotype, including during pregnancy.

#### P03.009

#### Cytogenetic changes of individuals occupationally exposed with arsenic

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Long-term exposure to inorganic arsenic from various factor has been documented to induce cancers and vascular diseases in a dose response relationship. Arsenic levels in urine, hair, and nail are biomarkers for short-term internal dose, skin hyperpigmentation and palmo-plantar hyperkeratosis are for long-term (many years) internal dose, and percentage of monomethylarsonic acid in total metabolites of inorganic arsenic in urine may be considered as an exposure marker for biologically effective dose. The biomarkers of early biological effects of ingested inorganic arsenic included blood levels of reactive oxidants and anti-oxidant capacity, genetic expression of inflammatory molecules, as well as cytogenetic changes of peripheral lymphocytes. In this regard present study find out whether the arsenic induced genetic damage or not in peripheral blood lymphocytes in exposed subjects. By

use of metaphase analysis with conventional Giemsa-staining, present study investigated both the groups. After signing a consent form, volunteers provided blood samples (5 ml) to establish cell cultures at 72 h. For karyotyping, minimum 40 complete metaphase cells from each subject was evaluated. Higher degree of chromosomal -type and chromatid type aberrations were observed in experimental compared to controls. Present study shown that the arsenic caused an increase in CA. The mean frequencies per 100 metaphase of major CA type (chromosome rings, translocations, and dicentrics) of the workers and the non-exposed controls were 0.91 and 0.24 respectively. Gene-gene and gene-environment interactions are involved in arsenic-induced health hazards through toxicological mechanisms including genomic instability and oxidative stress.

#### P03.010

##### Cytogenetic analysis in BOH cases- an Indian experience

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Chromosomal imbalance has been identified as a major cause for spontaneous pregnancy loss, infertility and childhood disability thereby, contributing significantly to the genetic burden on society. The diagnosis of such chromosomal anomalies in cases with bad obstetric history (BOH) can be made by conventional cytogenetics which involves karyotyping of GTG banded chromosome preparations obtained from whole blood. In the present study, conventional cytogenetic analysis was used to analyze peripheral blood samples from 167 individuals with BOH. Of these 167 cases, 145 presented with a history of spontaneous abortions (mean of 2 abortions) that were mainly in the first trimester. The remaining 22 cases included 20 with a history of children with congenital anomalies and 2 with death of children due to unknown causes. Cytogenetic analysis revealed chromosomal abnormalities in 10 cases. Robertsonian translocation between chromosomes 13 and 14 was reported in one case. Two cases revealed pericentric inversions in chromosomes 3 and 9 respectively. Novel cytogenetic anomalies like t(9;20) and t(1;13) were also observed in two cases. Three cases revealed heteromorphic variants in chromosome 1 (n=1) and 9 (n=2). Another case revealed addition of genetic material in the short arm of chromosome 15 that has not yet been reported in literature, to the best of our knowledge. The detection of these chromosomal anomalies, few of which are novel, in cases with BOH reiterates that cytogenetic analysis is a 'gold standard' for screening cases with BOH where structural anomalies are observed more frequently than aneuploidies.

#### P03.011

##### Identification of copy number variants associated with BPES-like phenotypes

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In 9 out of 27 patients (33%) CNVs not previously described as polymorphisms were detected. Four of these patients displayed psychomotor retardation as an additional clinical characteristic. In conclusion, we demonstrate that BPES-like phenotypes are frequently caused by CNVs, and we emphasize the importance of whole-genome copy number screening to identify the underlying genetic causes of these phenotypes.

#### P03.012

##### Mapping of Candidate Regions and Genes for Congenital Anomalies of the Kidneys and Urinary Tract (CAKUT) using Array-Based Comparative Genomic Hybridization

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<sup>1</sup>Institute of Human Genetics, Rheinische Friedrich-Wilhelms-University, Bonn, Bonn, Germany, <sup>2</sup>Division of Pediatric Nephrology, University Children's Hospital, University of Heidelberg, Heidelberg, Germany, <sup>3</sup>Department of Molecular Genetics, German Cancer Research Center, Heidelberg, Heidelberg, Germany. Congenital anomalies of the kidneys and urinary tract (CAKUT) are frequently associated with malformations of other organs. The etiology of maldevelopment often remains unknown. Therefore, we wanted to identify novel genomic regions associated with the CAKUT phenotype. We analyzed 30 unexplained CAKUT-patients with at least one additional extrarenal symptom using genome-wide array-CGH. In 3 patients, causal imbalances were detected. Patient HD1 was affected by the CAKUT-phenotype of hypospadias in addition to extrarenal anomalies. In the patient and his brother with a similar phenotype, array-CGH detected a terminal loss of 0.59Mb in chromosomal band 1q44 and a terminal gain of 6.55Mb in 16q23.3-q24.3 due to an unbalanced 1;16-translocation according to FISH-analysis. A balanced 1;16-translocation was detected in both patients' unaffected father. In patient HD16 presenting with renal hypoplasia and proximal ureteral stenosis in addition to mental retardation, macrocephaly, atresia of the auditory canal, and microtia, array-CGH detected a gain of 2.4Mb in 1q21.1. In the patient's unaffected father, a gain of 1.3Mb in 1q21.1-q21.2 was found involving the distal part of the patient's gain, for which benign copy number variation was described. In patient HD24 affected by renal dysplasia with hydronephrosis and extrarenal abnormalities, array-CGH identified a loss of 11.93Mb in 3q23-q25.1, confirmed and shown to be de novo by FISH-analysis. In summary, our study provides evidence that 1q44-loss and/or 16q23.3-q24.3-gain may be associated with hypospadias development, a uniallelic deletion in 3q23-q25.1 can cause renal dysplasia and hydronephrosis and that a renal phenotype may be associated with the 1q21.1-duplication genomic disorder.

#### P03.013

##### Cytogenetic study of tannery industry workers exposed to chromium compounds

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Chromium (Cr) is a metallic element which is listed by the Environmental Protection Agency as one of 129 priority pollutants. Chromium is considered one of the 14 most noxious heavy metals. Of the 1,083 tanneries in India, more than half, i.e. 577 are in Tamilnadu and of the 577, Chennai City and the North Arcot district account for as many as 397 tanneries. The production in Tamil Nadu is 44% of the total all-India production. Hence, the present investigation has been carried out the 4 regions in Tamilnadu namely Vanniampadi; Rani pet; Ambur and Erode. The objective of this study is to investigate the relationship between Cr workers and chromosomal alteration in the above population. In the present study totally 82 samples including 41 experimentals and 41 controls were selected. After signing a consent form, volunteers provided blood samples (5 ml) to establish cell cultures at 72 h. For karyotyping, 40 complete metaphase cells from each subject were evaluated. Higher degree of chromosomal and chromatid type aberrations were observed in experimental compared to controls. Statistically significant results were obtained with the value ( $P<0.001$ ), confirmed by Mann-Whitney U.

The present study concludes that Cr workers in tannery workers were found to have a higher level of CA; and the present study recommend that further research be conducted on the cellular mechanisms that lead to cancer due to exposure to different Cr valences.

## P03.014

**Estimation of interindividual variability of chromosomal radiosensitivity of human lymphocytes****N. Ryabchenko;**

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Increased chromosomal radiosensitivity of human lymphocytes was shown to be a genetically determined and is seemed to be a parameter of increased individual radiation and cancer risk. The study presents analysis of variations of chromosomal aberrations levels in human peripheral blood lymphocytes, induced by radiation in different cell cycle stages.

Blood samples from 114 healthy individuals were testified for G2 chromosomal radiosensitivity and 24 from them were testified additionally for G0-, G1-, and S- phase radiosensitivity. The dose of test gamma-irradiation of cell cultures was 1,5 Gy. Metaphase analysis was carried out in first mitosis.

Cytogenetic parameters (chromosomal breaks and gaps) in lymphocytes induced by test irradiation at G2-phase revealed the highest interindividual variability: coefficient of variation of 24% against 10 % at G0, 12 % at G1 and 15 % at S - phases of cell cycle. It was shown that distribution of cytogenetic parameters induced by test radiation at G2 phase did not correspond to normal and had bimodal character. Two groups of donors - with normal (88,4 % of samples) and increased (11,6 %) G2 radiation sensitivity on chromosomal level were revealed. There was strong correlation between S- and G2 chromosomal sensitivity ( $r^2= 0,88$ ) and G0 and G1 cytogenetic parameters ( $r^2= 0,76$ ). Correlation between spontaneous, G0 from the one hand and G2 chromosomal radiosensitivity was not revealed ( $r^2= 0,07$  and  $r^2= 0,11$ ). The results obtained testify for different genetics factors responsible for the formation of chromosomal radiosensitivity of human lymphocytes during the cell cycle.

## P03.015

**Cytogenetic and fish studies in patients referred for suspected chromosomal aberrations****I. Petkovic, I. Barisic;**

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Constitutional chromosome aberrations(CCA) cause a large group of genetic disorders and cytogenetic methods are important tool in diagnostics. Although cytogenetic techniques improved rapidly, the frequency of chromosomal aberrations and diagnostic strategy remain important medical problems.

We present the results of cytogenetic and FISH studies in 170 children referred to the Department of Medical Genetics for developmental and somatic delay, congenital malformations, dysmorphic features or behavioral problem of unknown etiology (common aneuploidy syndromes were excluded). The aim of this study was to determine the frequency of structural aberrations and evaluate diagnostic algorithm that starts with a clinical examination, followed by cytogenetic and FISH analysis.

Cytogenetic analysis was carried out on slides obtained by peripheral blood culture using high resolution GTG-, RBG- and CBG-banding method. FISH studies were performed using WCP-, CEP-, LSI-probes and subtelomere FISH screening assay.

Cytogenetic analysis revealed structural chromosome aberrations in 12(7.1%) out of 170 children. FISH analyses with appropriate micro-deletion probes were performed in 127 out of remaining 158 patients and microdeletions were identified in 14(11.0%) cases. Rearrangements involving subtelomeric regions were detected in 2(6.5%) out of 31 screened children. In our sample we identified 28(16.5%) children with structural aberrations including 23(13.5%) children with unbalanced rearrangements [dup(16); dup(8); der(15)t(15;18); der(8)t(7;8); der(X)t(X;6); del(2); del(14); r(9); psu idic(X) and 14 interstitial microdeletions], 3(1.8%) with apparently balanced aberrations [inv(13); t(6;17); t(6;10)], and 2(1.2%) with complex apparently balanced abnormalities [t(1;4),t(2;14),inv(3); t(6;19),t(2;13)].

This study demonstrates the usefulness of applied diagnostic strategy in the identification of genome imbalances in patients with suspected CCA.

## P03.016

**Lower consanguinity rate among Aneuploid patients in Iran****M. Shariati, E. Daei, R. Pazouki, H. Abassi, R. Jafari;**

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Background: some of the recent reports from Middle Eastern and Islamic countries claim of a higher chromosomal abnormalities among consanguineous marriages .Our\* personal studies from 1965 when one of us \* started Cytogenetics studies in Iran and the region does not support these reports. Here we are reporting 1136 Aneuploid patients (45,47,48 and 49 chromosome individuals) karyotyped by one of us\* during 1995-2005. Structural and diploid abnormalities of the patients chromosomes are excluded from this report.

**Materials and Methods:**

Whole blood peripheral samples were cultured in McCoy,s 5A plus 20% FBS and 2% PHA-M(Gibco) and 70 hours cultures were harvested after 1-2 hours colcemid and ten minutes KCL (0,075M) treatments. 4-5 times fixed cells in Carnoy solution (A.Acetic-Methanol 1V/3V) were used and Air-dried slides were G-banded by trypsin at the 350-400 G-bands level and analyzed. Fifty well spread mitoses were counted, analyzed and seven abnormal mitosis were karyotyped using the standard ISCN 1995 nomenclature.

Results: From 1136 Aneuploid patients .i.e: Trisomy 21, 47,XXY , 48,XXX , 47,XXX 47,XYY ,45X ,Trisomy 18, trisomy 15, and trisomy 13 and their mosaics . only 56 Patients had consanguineous parents corresponding to only five% of the Aneuploidy of the chromosomes. This clearly shows that at least at the numerical abnormalities of the chromosomes there is not any strong relationship with consanguinity of parents and the abnormal child. Since in Iran the rate of consanguinity is much higher than our five % aneuploid patients. In Down syndrome the association is stronger around 8%.

## P03.017

**An updated map of the genomic copy number variants in patients with congenital eye malformations****I. Balikova<sup>1</sup>, T. de Ravel<sup>1</sup>, C. G. Ayuso<sup>2</sup>, C. Villaverde<sup>2</sup>, K. Devriendt<sup>1</sup>, J. Fryns<sup>1</sup>, J. R. Vermeesch<sup>1</sup>;**<sup>1</sup>Center for Human Genetics, Leuven, Belgium, <sup>2</sup>Fundacion Jimenez Diaz, Madrid, Spain.

Poor sight and blindness cause severe impairment of the quality of life and have important socio-economical consequences. Congenital ocular malformations (COM) often result in lack or reduced vision. However, only in a small subset of the patients the molecular cause can be currently identified.

We hypothesized that chromosomal deletions and duplications are frequent cause for COM. We analyzed 55 patients with variable eye anomalies by 244K Agilent array CGH and we identified 4 certainly causal aberrations. Deletions affecting - OTX2, PAX6, FOXC1 and COH1 genes were detected. Interestingly a region including COH1 described as a benign variant was found in a homozygous state to cause the autosomal recessive Cohen syndrome. We also identified 2 loci containing novel genes potentially involved in COM.

Our findings improve the diagnosis of patients with congenital eye anomalies. Moreover they enable the identification of novel genes important for the development of the eye.

## P03.018

**Younger fathers have a higher risk for children with chromosomal aneuploidies: a paradox but not unexpected result****R. Masood, M. Riegel, D. Niedrist, O. Kundert, A. Schinzel, B. Steiner;**  
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Background: The past decades have seen a remarkable shift in the demographics of childbearing in Western countries. Numerous studies showed an exponential increase of the risk for offspring with chromosomal aneuploidies for advanced maternal age, but most studies failed to demonstrate a paternal age effect. In contrast, recent studies demonstrated that the risk for miscarriages is highest if both partners are advanced in age.

Methods: In a retrospective study, we analyzed the parental ages from postnatal cases with trisomies 21 (N=606), 13 (N=62) and 18 (N=150). Additionally, we analyzed the parental ages from 1'932'927 live-borns from Switzerland (1979-2006). As the reproductive success is dependent on the maternal and paternal age, we dichotomized the couples into two distinct groups (group 1: mother's age  $\geq$  father's age; group

2: mother's age < father's age).

Findings: With increasing maternal age the proportion of younger fathers increases continuously from 0.65 % (mothers: 15-19 years) to 45.2 % (mothers: 45-49 y.) in the control population. This phenomenon is more pronounced in the study group with aneuploidies (15-19 y.: 10%; 45-49 y.: 64.7 %). The odds ratio for a child with aneuploidy is significantly higher in couples with younger fathers (mothers: 25-29 y.: 2.04; 30-34 y.: 1.73; 35-39 y.: 1.64; 40-44 y.: 2.27; for all  $p < 0.001$ ). These findings were further confirmed by regression analysis.

Interpretation: The paternal age has a highly significant effect on pregnancy outcome. In all maternal age groups, the couples with younger fathers have nearly double the risk for a child with aneuploidy.

#### P03.019

##### False-positive diagnoses of Down syndrome in Lithuania

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In Lithuania (with the population 3.4 m people) we have registered 641 alive Down syndrome (DS) patient - 374 children under 15 yrs of age and 267 adults. For 393 of them (61.3%) karyotype analysis in lymphocyte culture was made. The chromosomal investigation has confirmed the diagnosis of DS in 372 (94.6%) patients. In 19 (4.8%) patients was found normal karyotype. In two patients chromosome anomalies other than trisomy 21 were found. The karyotype in one 3 years old boy was 49,XXXXY. The boy was deeply mentally retarded and had mongolian eyeslit, epicanthus, hypertelorism, palmar simian crease, clinodactyly, arches in papilar patterns on all ten fingers, and other microanomalies typical for DS patients. The other chromosome patient without trisomy-21 was 29 yrs old mild mentally retarded woman in whose karyotype ring chromosome 9 was found. For both patients the physicians suspected DS. Therefore, in Lithuanian population false-positive diagnosis of DS was found in 5.3% of patients, i.e. two - three times rarely as compared with analogically investigations in other countries. We suppose that one of the reasons of such situation is the racial homogeneity of Lithuanian population presented almost exclusively by Caucasians. But in newborn Lithuanian babies the frequency of false- positive diagnoses arises up to 32.1 percent: in 43 among 134 patients with clinical diagnosis of DS the normal karyotype was found. The main clinical feature - mental retardation - cannot be recognized in infants, and neonatologists do send the patients for karyotype analysis due to isolated microanomalies.

#### P03.020

##### Folate-related gene polymorphisms and risk for Down syndrome among Russian women

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Down's syndrome represents the most common chromosomal aneuploidy with high clinical significance. At least 90% of cases of 21 chromosome nondisjunction are due to maternal meiotic errors. Except for increased age at conception and altered recombination, maternal risk factors for meiotic nondisjunction are not well established. It's known that deficiency in cellular folate and methyl donors have been associated with pericentromeric DNA hypomethylation, chromosomal instability and abnormal chromosome segregation. Attempts to find polymorphisms of maternal folate-related genes associated with risk for Down syndrome have, however, often been inconclusive in different populations. We have studied six SNPs of the methylenetetrahydrofolate reductase gene (MTHFR 677C>T (rs1801133) and 1298A>C (rs1801131)), methionine synthase gene (MTR 2756A>G (rs1805087)), methionine synthase reductase gene (MTRR 66A>G (rs1801394)), methylenetetrahydrofolate dehydrogenase gene (MTHFD1 1958G>A (rs2236225)) and DNA methyltransferase 3B gene (DNMT3B -149C>T (rs2424913)) among 55 mothers of children with full form of trisomy 21 and 100 matched control mothers from Tomsk (West Siberia, Russia). Prevalence of alleles and genotypes of 1958G>A MTHFD1 and -149C>T DNMT3B polymorphisms were estimated in the Russian population for the first time. The frequency of mutant allele 66G of MTRR gene was higher among case mothers than among control mothers (71% vs. 53%;  $P=0.005$ ). Down syndrome case mothers also were significantly more likely than controls to be homozygous for the MTRR 66GG genotype (49% vs. 25%; OR=2.89, 95%CI:1.39-6.00;  $P=0.005$ ).

In conclusion, 66G allele and 66GG genotype of MTRR gene are associated with an increased risk for Down syndrome among Russian women.

#### P03.021

##### Partial trisomy of chromosome 21 without Down Syndrome phenotype

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Trisomy of chromosome 21 is associated with Down syndrome (DS) - the most commonly known genetic cause of mental retardation.

We report two patients with partial trisomy and tetrasomy 21 without DS phenotype. Conventional chromosomal analysis revealed one extra copy of derivative chromosome 21 in their peripheral blood lymphocytes. FISH and DNA analyses identified duplicated locuses (D21Z, D21S1414,D21S1435) spanning from the centromere to the band 21q21.1. Nimblegen targeted chromosome 21 array specified the range of duplication from the centromere to the band 21q22.11 in the first case and the range of duplication and triplication from centromere to the bands 21q22.11 and 21q21.1 resp. Additional material was of maternal origin in both cases.

These findings confirm the conclusion of nonpresence of DS when the bands 21q22.2 and 21q22.3 (Down critical region) are not duplicated. Our patients had nonspecific phenotypes although some of their features such as sandal gaps, joint hyperlaxity and hypotonia are present in patients with DS especially when an extra copy of region D21S55 located on 21q22.2 or very proximal 21q22.3 is present. We did not prove a duplication of this region in our patients.

#### P03.022

##### A disposable "microfluidic" chip for diagnostic FISH screening of genetic diseases

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FISH represents a fundamental tool for the diagnosis of a great number of genetic disorders. Currently FISH is performed on a simple device, i.e. a glass slide; cells from patients are processed through manual execution, followed by acquisition and analysis of results: overall the protocol is complex, costly (i.e. fluorescent probe) and require specific expertise, preventing its widespread utilization as screening tool.

We have recently characterized cluster-assembled nanostructured TiOx coating (ns-TiOx) as a biocompatible material with the unique properties of promoting cell adhesion of adherent and not adherent haematopoietic cells.

In this work we present the engineering of ns-TiOx coating into a prototype disposable microfluidic chip on glass slide for FISH analysis: our device allows to perform FISH with minimal volume of cells and reagents (less than 0.5ul), dramatically reducing cost (more than tenfold) and cell number requirements, preventing contamination between cell samples; it can be configured to perform single or multiple assays/ slide, increasing the throughput, with minimal variations of the standard protocol and compatibility with common high resolution microscopy based detection methods.

We have validated the chip by simultaneous FISH detection of Sex Chromosomes in a panel of haematopoietic tumor cells and Peripheral Blood Mononuclear Cells, assessing its efficiency, robustness and cost effective performance; we expect that this device would be suitable for FISH based genetic screenings in research and clinical settings, in particular for diagnostic evaluation of onco-haematological malignancies.

#### P03.023

##### Oligonucleotide-based high-resolution cytogenetics by customized fluorescence in situ hybridization (FISH)

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Recent advancements in our understanding of the structural complexity within the human genome have created a need for finer scale cytogenetic visualization of chromosomes. Since fluorescence in situ hybridization (FISH) provides the necessary positional and visual understanding of chromosomal architecture to complement other technologies for the discovery and analysis of cytogenetic variation, we set out to improve FISH performance through the introduction of oligonucleotide-based DNA probes. Using chemically synthesized complex libraries of oligonucleotides as templates for target regions identified as being variant by array comparative genomic hybridization (aCGH), we directed our probes only at the most informative elements within these regions to achieve high specificity with maximum flexibility. Probe generation involved a simple PCR amplification step, followed by the introduction of fluorescent labels by chemical modification. The downstream hybridization workflow closely resembles those widely used for BAC-based FISH. Here, we report the robust and specific visualization of six different regions that could not be readily assayed by traditional BAC-based FISH previously, with our initial studies detecting regions as small as 23 kb. One of these regions contained a known benign copy number change near the telomere of chromosome 6 which appears to be amplified in a non-homologous region of another chromosome. Our results indicate that these chemically synthesized complex library FISH probes will allow for structural characterization of abnormal findings seen by high-resolution methods such as aCGH and DNA sequencing and further advance our understanding of copy number variation in the human genome.

**P03.024****Genotoxicity of formaldehyde in human chromosomes**

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Formaldehyde is a clear, colorless, volatile liquid which has a pungent odor and slightly irritating taste. It is useful for industry and medicine but it is the forbidding material for the vegetable and seafood preservation. When formaldehyde is accumulated in human body, it causes eyes and skin irritation, suffocation, chest pain and death. The heparinized blood from thirty healthy Thais were studied for the impact of formaldehyde genotoxicity through human lymphocyte culture and G- banding technic. The formaldehyde concentrations of 0.036, 0.072, 0.15, 0.3, 0.576, 0.8 and 1.152 mg/ml were used in this experiment. The results showed that the numbers of metaphase chromosomes in the 0.036 and 0.072 mg/ml formaldehyde concentration groups were different significantly comparing with the control group ( $P<0.05$ ). The numbers of metaphase chromosomes were decreased when the concentration of formaldehyde increased. Loss of the human chromosomes in group B, D, E were observed in the formaldehyde concentrations of 0.036 and 0.072 mg/ml groups. No metaphase chromosomes were observed in the formaldehyde concentrations of 0.15, 0.3, 0.576, 0.8 and 1.152 mg/ml groups. It can be concluded that formaldehyde effects to human genotoxicity. Loss of chromosome induced by formaldehyde may increase risk of carcinogenesis in human.

**P03.025****Correlation between fragile sites and the content of micronuclei in human peripheral lymphocytes of patients treated with a beta-blocker antihypertensive drug**

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The antihypertensive drug atenolol was found to induce chromosome loss, as detected in micronuclei in the peripheral lymphocytes of treated patients. The fundamental question of which chromosomes the micronuclei were derived from remained to be answered. Analysis of structural chromosomal aberrations (CAs) and fragile site (FS) expression were performed in this study. They revealed a significantly high

incidence of chromosomal aberrations (chromatid and chromosome breaks) in patients, where ten FS emerged as specific. Also, the band 17q12-21, where known fragile sites have not been reported, was only expressed in patients. Fluorescence in situ hybridization using chromosome-specific painting probes revealed the preferential inclusion of chromosomes 7, 11, 17 and X in micronuclei (MN) of patients. The results also suggest a correlation between chromosomal fragility and content of MN and support the findings for a linkage between hypertension and a locus on chromosome 17.

**P03.026****Increased unbalanced structural chromosome alterations in lymphocytes from fishermen who participated in the clean-up of the Prestige oil spill detected two years after their exposure**

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Until now the effects of genotoxicity due to fuel oil exposure have only been reported during initial acute exposition. The objective of the present study was to evaluate the persistence of genotoxic effects two years after the exposure on individuals who participated in the clean-up of the oil spill caused by the wrecked tanker Prestige. The fishermen, who were non-smokers and in good health, were included in exposed (E) group (>15 days of cleaning-up tasks at least four hours per day) and non-exposed (NE) group. A total of 91 E and 46 NE fishermen were studied. The collection of the samples was performed between July 2004 and February 2005. Cytogenetic damage was determined by structural chromosome alterations from peripheral blood lymphocytes. For each subject, more than 25 karyotypes were examined using 72 h cultures. Our results showed a positive association between frequency of structural chromosome alterations and fuel exposure (196 structural chromosome alterations in a total of 2448 karyotypes analyzed) vs NE (33 /1285)( $P<0.0001$ ). Of them, the unbalanced chromosome alterations (deletions, acentric fragments and marker chromosomes) were more frequently found in the E group (184 unbalanced structural alterations in a total of 196 structural chromosome alterations) vs NE (26/33) ( $P=0.01$ ). In conclusion, participation in clean-up tasks of oil spills results in prolonged genotoxic effects lasting two years after the exposure, however their human health consequences are unknown. Acknowledgments: This work was supported by FIS (PI07-0086) and CIRIT (2005, SGR-00495)

**P03.027****Cytogenetic analysis in 317 couples with recurrent spontaneous abortions**

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Recurrent spontaneous abortions (RSA) represent a common medical matter. RSA is defined by the WHO as two or more consecutive pregnancy losses prior to the 20th week of gestation. The etiology of RSA is complex and still not completely understood. A variety of factors underlie the occurrence of miscarriage - these include genetic factors (chromosomal abnormalities, genic mutations), external factors (drugs, ionising radiation, lead), infectious factors (viruses, bacteria); anatomic anomalies of the uterus (malformations, fibroma, cervical insufficiency); immunological factors; chronic maternal or paternal disease. Chromosomal abnormalities are thought to be the cause of about 50% of the miscarriages in the first trimester of pregnancy, of 5% of those in the second trimester and of around 0.5% of the new-borns. At chromosomal analysis of the couples with RSA, 2-5% of these were found to have one partner involved with an equilibrated chromosomal abnormality which is to lead to the occurrence of gametes with duplications or deficiencies of chromosome segments. Cytogenetic investigations were performed on 634 patients with reproductive problem using GTG-banding. The frequency of chromosomal abnormalities was 6%. Structural balanced aberrations of autosomal chromosomes were detected in 21 cases. Compared to published data, the observed chromosomal abnormality rate in the present study is considered similarly. Supported by a grant CNCSIS type A 546/2007, Ministry of Education and Research, Romania.

## P03.028

## HLA B27 allele status in turkish patients with Spondyloarthropathy

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Some of the spondyloarthropathies are inflammatory joint diseases of the vertebral column associated with the MHC class I molecule HLA-B27. It has shown that HLA-B27 heavy chains can form stable homodimers lacking β2m, and it is possible that such an aberrant form of the B27 molecule could be involved in disease pathogenesis

Two hundred and seventeen patients admitted to our center because of arthropathy and or uveitis symptoms between July 2008 and January 2009 were analysed for HLA B27 allele status. Patients were informed and gave consent. Genomic DNA was isolated from peripheral blood leukocytes by ethanol precipitation. HLA B27 allele status was searched by sequence specific primer method by multiplex PCR covering HLA B27 and B73 alleles. With the detection of the 145 and 95 base pair products along with internal control bands it was considered as the presence of these alleles.

In this study 67% (145/217) of our patients were carrying B27 allele. There were patients with uveitis (32/145), reactive arthritis (25/147), inflammatory bowel disease (18/147), spondyloarthropathy (72/147). Patients had neutrophilic type leukocytosis (43%), raised ESR (66%) and anemia (45%). We determined female predominance in allele positive group in contrast to the literature ( $p<0.05$ ). There was one patient who had HLA B27 allele and positive Rheumatic Factor.

As a conclusion we had confirmed that patients with a positive HLAB27 allele status had more prone to spondyloarthropathy. In our group, female patients specially middle aged females with sacroileitis must be subjected for B27 typing.

## P03.029

## Microdeletions seem a more common cause of holoprosencephaly than point mutations

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The holoprosencephaly (HPE) phenotypic spectrum can vary from a mild single median maxillary central incisor (SMMCI) to severe cyclopia. The etiology of HPE is heterogeneous with at least 20% of the cases presenting chromosomal anomalies. Mutations of the four major HPE genes explained another 20% of living infants with normal karyotype. Recently, gene dosage methods found large deletions in *SHH*, *ZIC2*, *SIX3* and *TGIF* in 8% of the cases (4% in liveborn children, and 12% in fetuses). We studied, mainly from the ECLAMC (Latin American Collaborative Study of Congenital Malformations) DNA Bank, 148 HPE, mostly liveborn infants, and 10 SMMCI cases without recognized chromosomal anomalies, finding 10 mutations on the main HPE genes (6%). Nineteen patients without mutations from 10 families with HPE, and 7 with SMMCI, and 6 "positive" controls for chromosomal anomalies, trisomy 13, del(18p), t(7;14), and inv(5), were screened for microdeletions or gains using multiplex ligation probe-dependent amplification (MLPA) with SALSA MLPA kit P187. For "positive" controls, only the three inv(5), from the same family, had no alterations using this kit. For patients without mutations or chromosomal anomalies we identified a microdeletion on *SHH* in a male patient with HPE, microcephaly, cyclopia, proboscis, microstomia, low set ears, short neck, bilateral single palmar crease, anal atresia and sacral agenesis. A loss of *ZIC2* was identified in a male patient with HPE, ocular hypotelorism, narrow palate, low neck hair and hirsutism. The microdeletions seems to be more common cause of HPE (12%) in younger patients than point mutations (6%).

## P03.030

## Nystagmus and 3-4 syndactyly of hands due to a 2q31.1 duplication involving the HOXD cluster

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Mutations or deletions of *HOXD* genes are known to induce limb anomalies such as synpolydactyly for instance when they involve *HOXD13*. Here we describe a father and his daughter referred to the genetic clinic for the association of bilateral 3/4 finger syndactyly and a nystagmus. Array-CGH was performed in both of them and revealed a 2q31.1q31.2 duplication of around 3.8Mb. This region comprises 27 genes and involves the whole *HOXD* cluster.

This is the first report of bilateral hand syndactyly associated with a duplication of *HOXD* genes.

Among the other involved genes, one of them seemed particularly interesting. Indeed, the duplication implicates *CHN1* which encodes two Rac-specific guanosine triphosphatase (GTPase)-activating α-chimaerin isoforms. *CHN1* belongs to the *DURS2* locus which is involved in autosomal dominant forms of Duane's retraction syndrome (DRS). Miyake et al. recently identified missense mutations of *CHN1* which induce a gain of function of alpha2-chimerin and cause aberrant innervations of the oculomotor muscles in animal models.

The cases we report exhibit a nystagmus, which differs from DRS. Since *CHN1* plays a major role in ocular motor axon pathfinding, we hypothesize that a duplication of this gene might be responsible for the nystagmus.

## P03.031

## 21-year chromosomal studies among the Iranian infertile women

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The infertility is an important health problem, affecting about 15% of couples. The important role of genetic factors in pathogenesis of infertility is now increasingly recognized. The value of karyotyping women in the routine work-out of couples referred for sterility has long been recommended. The aim of this study was to define the frequencies of all chromosomal aberrations in Iranian infertile women. In this 21-year retrospective study, we investigated 896 women which referred to our Department due to infertility between 1986 to 2006. For chromosome analysis, heparinized peripheral blood samples were cultured, harvested and banded according to standard methods. Out of 896 patients, 712 patients (79.46%) had a normal karyotype, 48 patients (5.36%) showed Turner's syndrome (45,X), and 44 patients (4.91%) were sex reversal with 46,XY Karyotype. The rest of 92 patients (10.27%) revealed a wide range of chromosome abnormalities which is shown in the following table.

Karyotype	Number	Per cent
46,XX	712	79.46
45,X	48	5.36
46,XY	44	4.91
mos45,X/46,XX	19	2.13
mos45,X/46,XY	5	0.56
mos46,X,i(X)(q10)/45,X	14	1.57
mos46,X,i(X)(q10)/46,XX	3	0.33
46,X,i(X)(q10)	11	1.23
47,XXX	4	0.45
46,X,del(X)(q)	8	0.89
46,X,del(X)(p)	3	0.33
46,X,r(X)	4	0.45
46,X,del(Y)/45,X	1	0.11
46,X,t(X;2)	1	0.11
46,X,t(19;X)	2	0.22
45,X,inv(1)	1	0.11
46,XX,inv(9)	4	0.45
46,XX,9qh+	1	0.11
46,X,+mar/45,X	5	0.56
47,XX,+mar	2	0.22
46,XX,psu dic(X;X)	3	0.33
47,X,-X,+fis(X)(p10);+fis(X)(q10)	1	0.11
<b>TOTAL</b>	<b>896</b>	<b>100</b>

**P03.032****Infertility Cytogenetic Causes in men****S. Soleimani;***The Blood Transfusion Organization research center, Tehran, Islamic Republic of Iran.*

Male infertility accounts for about 50% of infertility.

Some of the chromosomal changes (aberrations) detected in male infertility include:

1. Balanced chromosomal translocation
2. Chromosome inversion
3. Marker chromosome
4. Sex chromosome abnormality

Our investigation provides evidence confirming the importance of the sex chromosomes in reproductive disorders. We have analyzed, over 10 years of study, 1021 blood samples from infertile men, of whom 664 of them were oligospermic or azoospermic. Constitutional chromosome aberrations were diagnosed in 321 of these patients. We observed chromosomal abnormality in 31.4% of azoospermic men, similar to data from literature.

The following abnormal karyotypes were found:

- 46,XX;47XXY;47,XYY;48,XXXYY;45,X[10]/46,XY[134];46,XY[4]/47,XXY[82];  
 46,XX[11]/47,XXY[36];46,XY[6]/47,XYY[38];46,XY[10]/46,XX[26]/47,XXY[61];  
 46,X,del(Y)(q<sub>11..23</sub>);46,X,inv(Y)(p<sub>11..2</sub>;q<sub>11..22</sub>).

We found some patients with complex structural and aneuploidy abnormalities:

- × 46,XX,inv(9)(p<sub>11..13</sub>)/47,XXY,inv(9)(p<sub>11..13</sub>)[4]
- × 47,XXY[93]/48,XXY+mar[4]/48,XXXYY[2]
- × 47,XXY,inv(9)(p<sub>11..13</sub>)
- × 47,XXY,t(1;17)(p<sub>36..1..21</sub>)
- × 46,X,del(Y)(q<sub>11..2</sub>)[98]/45,X[6]
- × 47,XXY,inv(9)(p<sub>11..13</sub>)/t(10;22)(q<sub>26..3..13..1</sub>)
- × 46,X,idic(Y)(p<sub>11..32..1..13..32</sub>)[27]/45,X[36]/46,XY[2]

We believe that many infertile men, especially severe oligospermic and azoospermic cases, require cytogenetic analysis to reveal the existence of gonosomal abnormalities.

**P03.033****Cytogenetics study of 103 Intellectually Handicapped School Children in Iran.****M. Shariaty, E. Daei, H. Azin, I. Nabipour, H. Abousaidi, H. Abbasi;***Cancer research institute, Tehran, Islamic Republic of Iran.*

Mental Retardation is a medically and socially important health issue affecting 2-3% of the general population. It is a multifactorial entity in which genetic abnormalities at the chromosomal or molecular level are a major etiological cause. A review of literature indicates that chromosomal abnormalities are the cause of up to 30% of all mental retardation. The present study investigated The frequency of major chromosomal abnormalities in 103 pupils of special educational schools in Bushehr province on the north shore of the Persian Gulf. The children were twelve to eighteen years old from three special schools of Bushehr.

**Materials & Methods:** Peripheral blood samples of the boys and girls were obtained and analyzed using the standard 70 hours lymphocyte culture technique. McCoy's 5a medium plus FCS and PHA was used. After 2 hours colcemid treatment and 10 minutes KCL (0.075 M) treatment fixation and slide preparation 50 well spread G-banded mitoses at the 350-400 band level were analyzed and seven abnormal mitoses were karyotyped and reported using ISCN- 1995 nomenclature.

**Results:** a total of 23 pupils had major chromosomal abnormalities detectable at that resolution :16 persons had Trisomy 21, two had fragile X syndrome, one was mosaic 48,XXXYY/47,XXY, one had ring chromosome number 3 and two had structural aberrations of chromosomes 5 (5q deletion) and chromosome 13(13q 2.31-2 duplication).

**Conclusion:** Twenty- three per cent of our pupils (with an IQ of 50-70 ) studied in Bushehr had a major chromosomal abnormality of which the commonest were Trisomy 21 and Fragil X syndrome.

**P03.034****Cytogenetic abnormalities found among the females suspected for mental retardation in and around Coimbatore city, Tamilnadu, India****K. Sasikala, V. Balachandar, P. Manikantan, S. Mohanadevi, R. Sangeetha;**  
*Bharathiar University, Coimbatore, India.*

Mental retardation is a condition, not a disease, nor should it be confused with mental illness and is an important health problem all over the world. The prime aim of the present study was to identify the chromosomal alterations in females with mentally retarded patients in Coimbatore region. In order to investigate the possible cytogenetic damage to the mentally retarded patients, a G-banding method was carried out on the lymphocytes of 106 female experimental and equal number of female controls selected. Experimental and controls were selected based on the detailed questionnaire. In the present study volunteers provided blood samples (5 ml) to establish cell cultures at 72 h. For karyotyping, 40 complete metaphase cells from each subject were evaluated. The detection of an increasing number of recognizable syndromes such as Downs syndrome, Turner syndrome associated with chromosomal anomalies 45,XO; 47, XXY; 46, XX+21; 46,XX,15s<sup>+</sup>; 46, XX,21s<sup>+</sup>; 45 ,XO/46,XX, 46, XX,13s<sup>+</sup>; 45,XO/47,XXX; 45,XO/46,XX,r21; 46,XXt (13q; 21q<sup>+</sup>) was found among the female of the city through the chromosomal analysis of the subjects. In the present study exhibit chromosomal aberrations showed higher degree in experimental compared to controls ( $P<0.001$ ).

In conclusion, to point out that, the systematic study of mental retardation is very rewarding with an accurate diagnosis in the proband and with proper genetic counseling, one can alleviate the misery of many parent and also can reduce the birth of affected offspring's in the family subsequently.

**P03.035****A new Y inversion in a man: Case Report****M. Zamaniyan, F. Manoochehri, F. Mortezapour, F. Nasiri, M. Rahnama, F. Razazian, F. Mahjoobi;***Iranian blood transfusion organization, Tehran, Islamic Republic of Iran.*

A man was referred to us for chromosomal analysis because of infertility.

**Material & Methods:** Lymphocyte cultures from the patients were set up in RPMI1640 supplemented with 20% FBS. High resolution chromosome banding was performed.

**Result:** The karyotype was assessed as 46, X, inv(Y) (q11.2; 12).

**Discussion:** Karyotyping was recommended for his brother and his father, and was normal. The infertility present in this man could be caused by the possible deletion of important genes located at the break points or it could just be a coincidence.

**P03.036****A novel case of partial trisomy 2p in a 2-year old girl****S. Unlubay<sup>1</sup>, O. Cogulu<sup>2</sup>, B. Durmaz<sup>2</sup>, A. Alpman<sup>1</sup>, F. Ozkinay<sup>2</sup>;**<sup>1</sup>Ege University Faculty of Medicine Department of Medical Genetics, Izmir,<sup>2</sup>Ege University Faculty of Medicine Department of Pediatrics, Izmir, Turkey.

The characteristics of partial duplication of the short arm of chromosome 2 have been documented in different reports. The clinical phenotype of trisomy 2p includes growth and psychomotor retardation, microcephaly, prominent forehead, hypertelorism, epicanthal folds, ptosis, strabismus, myopia, apparently low set and abnormal ears, flat nasal bridge, narrow high palate, micrognathia, sternal abnormalities, epileptic seizures, kyphoscoliosis, congenital heart disease, genital hypoplasia, long widely spaced fingers and toes and hypotonia. Here, we report on a new case of inv dup 2p in a 2 year-old girl. She was born to no non-consanguineous parents after an uncomplicated pregnancy. The birth weight was 2.800 kg. The physical examination revealed generalized hypotonia, pectus excavatum, frontal bossing, flat nasal bridge, hypertelorism, low-set and large ears, micrognathia, syndactyly in the 2-3-4 toes. She also had severe growth and psychomotor retardation. At 6 months, she had the first epileptic seizure and three more seizures occurred afterwards. The EEG showed abnormal discharges. She also had recurrent respiratory infections. Her thorax CT scan showed subsegmental atelectasis in the posterior lobes and areas of bronchiolitis obliterans. Gastroesophageal reflux was determined in the scintigraphy. The cranial MRI was normal. The

karyotype revealed a partial trisomy of chromosome 2p and was noted as 46,XX,inv dup(2)(p23p25.2). The parents had normal karyotypes. According to the clinical picture and the karyotype, the patient was considered to be a novel case of partial trisomy 2p and fluorescence in situ hybridization (FISH) and array comparative genomic hybridisation (aCGH) studies were planned for revealing the exact breakpoints.

#### P03.037

##### Abnormal karyotype rate in men from infertile couples

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Assisted reproductive technologies (ARTs) allows to overcome infertility. Many cases of idiopathic infertility due to numerical and structural chromosome abnormalities result in unbalanced karyotype in offspring.

We aimed at evaluation of abnormal karyotype rate in men from infertile couples. The examined cluster totaled 113 individuals. Patients were subdivided into 6 groups according results of semen analysis (WHO criteria): 15 azoospermia, 43 oligoasthenoteratozoospermia (OAT), 4 oligoteratozoospermia (OT), 39 asthenoteratozoospermia (AT), 1 asthenozoospermia (A), 11 teratozoospermia (T). Karyotyping, performed on QFH-banded metaphase chromosomes from peripheral blood lymphocytes, revealed chromosome abnormalities in 8,84% of subjects. Two chromosome aberrations were detected in azoospermia group: 46,X,t(X;Y) and 45,X[25]/46,XderY[75]. 6 abnormal karyotypes with 3 of them as robertsonian translocations carriers: 45,XY,der(13;14)(q10;q10) were detected in individuals with low spermatozoa count (OAT and OT). One abnormal chromosome have been registered in men with AT Chromosome polymorphism was detected in 17% among OAT and OT men and in 25,64% in AT men.

Our results prove the importance of karyotyping of men nominated for ARTs

#### P03.038

##### The clinical effects of isochromosome Xq in Klinefelter Syndrome: Report of a case and review of literature

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We describe a male with a variant Klinefelter syndrome, and trisomy Xq resulting from an isochromosome Xq [47,Xi(Xq)Y]. He had many characteristics of classical KS: bilateral atrophic testes and microcalcifications, normal masculinization, azoospermia, hipergonadotropic hypogonadism elevated FSH and LH, normal intelligence, microcalcifications and normal androgenization, but his stature was not increased. Ultrasonographic evaluation also revealed parenchymal alterations secondary to previous epididymo-orchitis. After initial evaluation the patient underwent incisional biopsy of testes which showed tubular hyalinisation, Leydig cell hyperplasia and sertoli cell only syndrome. The i(Xq) found in all cells analyzed. These findings indicate that extra copies of the long arm of X have phenotypic expression, even though activated only in early development. In conclusion, review of literature on 20 adult patients support the view that the presence of an isochromosome Xq in KS has a favorable prognosis in terms of normal mental development and normal stature. This case provides an opportunity to study the effect of the isochromosome Xq on the phenotype.

#### P03.039

##### Chromosomal alterations and Micronuclei frequencies in smokers who were occupationally exposed to lead in Tamilnadu region, India

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Lead poisoning is an environmental and public health hazard of global proportions also tobacco smoking is the main cause of mortality worldwide. The focal aim of the present study was to identify the CA and MN frequencies in cigarette smokers chronically exposed to lead. For this purpose, totally 39 smokers who were worked with lead based

industries and 39 controls were selected. By use of metaphase analysis with conventional Giemsa-staining, present study investigated both the groups. After signing a consent form, volunteers provided blood samples (5 ml) to establish cell cultures at 72 h.

The lymphocyte count in the Experimental, except Group I of printing press and Group III of petrol sniffing subjects exhibited elevated values when compared with those of the Controls. The increase in values of Experimental was statistically significant. MN frequency was higher in exposed workers than in controls and smoking status significantly raised MN frequency among the exposed workers but not among controls. In the present study, the incidence of MN in peripheral lymphocytes from workers exposed to smoking was over twice as high in the controls ( $P<0.001$ ).

It may be concluded that the workers occupationally exposed to lead toxicity, had significant haemotoxic and genotoxic changes which need to be elucidated. The reason was not only to protect the genetic material of future generations but also in the prevention of malignant diseases in the present/future populations working in the autoguarage, printing press and petrol sniffing as well as those residing in the industrial areas.

#### P03.040

##### De novo interstitial 15q deletion identified by *FBN1* MLPA and refined by array-CGH in a female teenager with an incomplete Marfan syndrome

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Interstitial deletions involving the 15q21.1 band are very rare. Only 4 of these cases have been studied using molecular cytogenetic techniques in order to confirm the deletion of the *FBN1* gene. The presence of clinical features of the Marfan syndrome (MFS) spectrum associated to mental retardation has been described in only 2/4 patients. Here we report on a 16-year-old female referred for suspicion of Marfan syndrome (positive thumb and wrist sign, scoliosis, joint hyperlaxity, high-arched palate with dental crowding, typical dysmorphism, mitral insufficiency with dystrophic valve, significant striae). There was no family history of MFS. She had therefore 3 minor criteria according to the Ghent nosology. She also had complex dyslexia but could follow normal school training. Direct sequencing of the *FBN1*, *TGFBR1* and *TGFBR2* genes was negative. MLPA revealed a genomic deletion of the whole *FBN1* gene, confirmed by multiplex semi-quantitative PCR. The deletion was confirmed by FISH and was not found in the parents. Array-CGH permitted to define a 2.97 Mb deletion including 19 genes. This case is the smallest microdeletion including *FBN1*. Contrary to the other published observations, our proband do not exhibit mental retardation. Haploinsufficiency of *FBN1* is likely to contribute to the presence of features within the MFS spectrum. However, attenuated features could be explained because disturbances of TGF-beta signalling associated with *FBN1* mutations do not exert full phenotypic effect through simple haploinsufficiency. Phenotypic variability with other patients with interstitial deletions including 15q21.1 band may reflect differences in deletion size and/or in-trans modifying factors.

#### P03.041

##### Micronuclei in human lymphocytes exposed to sodium pertechnetate, *in vitro*

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Technetium-99m (<sup>99m</sup>Tc) has become the most widely used radioisotope in the detection of inflammatory sites as well as in the diagnosis of transplanted tissues. The goal of the present work is to study the effect of sodium pertechnetate on human lymphocytes *in vitro*, using the micronucleus assay. Micronuclei appear during cell division as a result of acentric chromosome fragments or whole chromosomes, outside the nucleus. Peripheral whole blood cells collected from healthy donors, 18 to 30 years old, were incubated at 37°C for 48 hours in the presence of <sup>99m</sup>Tc (3.7 MBq/100μl). Cells not exposed to the radionuclide served as control for the experiment. Cytochalasin B (3μg/ml) was added to the cultures 20 h postinitiation. After fixation, cells were stained with

Giemsa Gurr (2%) and were observed under optical microscope. In the test group, 12077 binucleated cells were studied and 212 of them showed alterations ( 18 micronuclei and 194 nucleoplasmic bridges). Nucleoplasmic bridges are biomarkers of genomic instability and are a result of chromosomal rearrangements involving more than one centromere (dicentric chromosomes). In the control group 12056 cells were observed and 06 micronuclei were seen. The chi-square test with Yates correction indicated that the results were extremely significant ( $p<0.0001$ ) suggesting that sodium pertechnetate was responsible for the chromosome alterations observed.

#### P03.042

#### Genotoxicity evaluation in chronic renal patients undergoing hemodialysis and peritoneal dialysis, using the micronucleus test

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Chronic renal patients on hemodialysis (HD) and peritoneal dialysis (PD) treatment are exposed to oxidative stress and DNA damage. Therefore, cancer incidence and genomic damage of peripheral lymphocytes are elevated in patients with end-stage renal failure. The genotoxic effect may cause the loss of chromosome fragments, or even entire chromosomes, which form micronuclei after cell division, and can be detected by the micronucleus test, a sensitive and reliable system for the evaluation of spontaneous and mutagen-induced DNA. In the present case-control study, we evaluated the genotoxic effect in the peripheral lymphocytes of 65 patients undergoing hemodialysis, and 61 subjected to peritoneal dialysis, matched for gender and age with 60 controls. MN frequency was expressed as the number of micronuclei per 1000 binucleated cells. Our results revealed that patients undergoing hemodialysis ( $7.1 \pm 4.4$ ,  $P=0.006$ ) and peritoneal dialysis ( $6.6 \pm 4.8$ ,  $P<0.001$ ) treatment have a significantly higher frequency of micronucleated cells compared to control subjects ( $4.5 \pm 2.6$ ). However, there was not a statistically significant difference between MN levels of hemodialysis and peritoneal dialysis patients ( $P=0.11$ ). In conclusion, treatments such as HD and PD induce oxidative stress and consequent oxidative damage.

#### P03.043

#### Mustard Gas Long Term Effects on Chromosomes of Persian Soldiers Exposed During 1980-1988 Iran-Iraq war

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Mustard Gas (MG) is a strong alkylating toxic vesicant gas with an LD<sub>50</sub> of 1500 mg. This very lethal gas was used during world wars and local wars against army and civilian personal as a mass destructive weapon with thousands death and millions of injuries. Long term adverse effects of MG on human cells has not been studied in the last four decades. So our knowledge of its long term effects on human population was scanty.

Unfortunate Iraq-Iran war and the mass usage of MG by Iraqi's Army against thousands Persians provided us the opportunity of studying MG long term effects on the chromosomes of exposed soldiers.

#### Material and Methods:

Peripheral leukocytes of 115 exposed males aged 32-76 after 14-20 years from contamination and 30 unexposed matched control males were cultured in McCoy's 5A plus 20% FBS and 2% PHA-M (GIBCO) and harvested in the 3rd day after one hour colcemide treatment and ten minutes KCL (0.075 M) shock. 4-5 times changed carnoy fixative, Air-dried slides were G-banded and Analyzed by an experienced cytogeneticist \* and rechecked.

Results: 101 exposed and all 30 controls resulted in good quality mitoses. Seven abnormal mitosis were karyotyped. 31 per cent of the exposed and none of the control group had at least one break .Also

marker chromosome in two cases and missing Y chromosome in 3 cases were observed. These findings indicate the long term cytogenetics effects of MG on chromosomes of somatic cells of exposed individuals. We are studing MG effects on germ cells.

#### P03.044

#### Cytogenetic studies among Plant Nursery Workers occupationally exposed to pesticides

S. Mohanadevi, V. Balachandar, P. Manikantan, R. Sangeetha, K. Sasikala; Bharathiar University, Coimbatore, India.

Pesticides are among the most widely used chemicals through out the world. They include a great variety of substance different both in composition and properties with the purpose to kill, destroy or repel undesirable living organisms. It is estimated that nearly 10,000 deaths annually to use of chemical pesticide worldwide, with about three fourths of these occurring in developing countries. The chromosomal aberrations (CA) assay is important for monitoring the populations exposed to genotoxic agents because it allows the evaluation of the entire genome to identify mutagenic and carcinogenic chemicals. Hence the present study as an eye opener to health risks associated with prolonged low doses of pesticide exposure in such workers and emphasizes the need for awareness among such workers as far as pesticide handling concerned. Find out the genetic effects by mixed pesticide chemicals and ingredients. In the present study chromosomal aberrations namely chromatid gaps and breaks, chromosomal gaps, breaks, deletions and translocations were higher in experimental compared to the control samples. Also the frequency of chromatid aberrations was higher when compared to the chromosomal aberrations ( $P<0.001$ ). All results of the present study indicate that the level of cytogenetic damage was significantly affected by the pesticide exposure of subjects. Another explanation for the genotoxic damage observed is the lack of protective measures taken by the workers. Therefore, there is a need to educate those who work with pesticides about the potential hazard of occupational exposure and the importance of using protective measures.

#### P03.045

#### Aneugenic and clastogenic components of the cytogenetic effects of incorporated plutonium-239

S. A. Vasilyev, V. A. Timoshevsky, I. N. Lebedev;

Scientific Research Institute of Medical Genetics, Tomsk, Russian Federation. Genetic monitoring has been traditionally used for the surveillance of populations exposed to environmental mutagens including ionizing radiation, chemicals and life-style factors, and medical treatments. Among the consequences of mutagenic exposure aneuploidy is the most important one because of great number of genes involved in these abnormalities. Unfortunately, aneugenic potential of ionizing radiation especially *in vivo* is poorly understood. Therefore we have analyzed effects of ionizing radiation on 20 male workers of Siberian chemical plant with activity of incorporated plutonium-239 from 10 to 188 nCi by means of FISH using human centromeric DNA probes in binucleated lymphocytes. We have used group of 20 health male individuals living in Seversk and having no connection with ionizing radiation as a controls. There was a significant increase of the overall micronuclei frequency in the exposed group (8.6 %) as compared with the controls (5.1 %,  $p<0.001$ ). This increase mostly was due to higher frequency of centromere-negative micronuclei (MnC-) in the group of workers (4.7 %) than in the controls (2.4 %,  $p<0.001$ ). But also significant increase in the centromere-positive micronuclei (MnC+) frequency was observed in the exposed group (3.9 %) compared with the controls (2.7 %,  $p<0.05$ ). For the first time it was confirmed that the influence of complex of occupational factors including incorporated plutonium-239 in addition to clastogenic effect (MnC-) results in increase of hypoploidy frequency (MnC+).

#### P03.046

#### Chromosomal abnormalities in prenatal diagnosis.

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Chromosome abnormalities are one of the major causes of first trimester pregnancy loss and birth defects. The most frequent autosomal aneuploidies are: trisomies 21 (the incidence of Down syndrome is estimated at 1 per 700 births - 0.14%), 18 (the incidence of Edwards syn-

drome is estimated at 1 per 5000 births - 0,02%) and 13 (the incidence of Patau syndrome is estimated at 1 per 10000 births - 0,01%). Amniotic fluid karyotyping and FISH (Fluorescent In Situ Hybridization) have been offered to pregnant women with genetic risk, using the standard method and GTG banding techniques.

This study presents the cytogenetic results from a total of 654 cases that have been analyzed between 2008 - 2009. We have found 18 abnormal karyotypes of which 16 cases have been numerical abnormalities (13 homogeneous aneuploidies: trisomies - 3 cases of 47,XX+21, 5 cases of 47,XY+21; 4 cases of 47,XY+18 and monosomies - 2 cases of 45,X0; 1 pseudomosaicism - 47,XX+2 and 1 triploidy - 69 XXX), 2 structural abnormalities (one case of 45,XX with translocation 45,XX, t(13q;14q) and one case of 46, XY, der(14;21)(q10;q10) +21) and 11 normal variants (7 cases of 46, XX inv(9)(p11;q13); 2 cases of 46,XY inv (3)(p11;q11.2); one case of 46,XX inv(3)(p11; q11.2), one case of 46,XY inv (3)(p11;q11.2) inv(9)(p11;q13)).

The numerical abnormalities have been further verified by FISH analysis. This report confirms the importance of karyotype and FISH in prenatal diagnosis.

#### P03.047

#### Not so irrelevant pseudomosaicism of trisomy 18 previously found in amniotic fluid culture

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In 1993, a woman (G4, P3) underwent amniocentesis because of maternal age. Regular chromosome analysis resulted in thirteen colonies showing a normal female karyotype and a single isolated metaphase with 47,XX,+18. After extensive work-up according to international prenatal quality standards, no other colony with trisomy 18 was found in a total of 29 colonies. Therefore, the finding of a single "47,XX,+18" cell was considered to represent a pseudomosaicism and the foetus was diagnosed with a normal female karyotype.

Fifteen years later and at the age of 14, the very same girl was referred to our department because of mental retardation and autistic-like behaviour. After exclusion of a (pre)mutation for Fragile-X syndrome, oligo array CGH analysis was performed, using the Agilent 105 K Oxford array (custom design ID: 019015), in search for a previously undetected (sub)microscopic genomic imbalance.

To our surprise, a slightly elevated ratio was found for all (2424) oligo probes on chromosome 18, suggestive for a mosaic pattern of trisomy 18. No further genomic aberrations were detected. Subsequent FISH analysis on interphase and metaphase cells of blood and other tissue samples from the patient confirmed the presence of a mosaic pattern for trisomy 18.

This case demonstrates that, even after careful cytogenetic examination, a "with-near-certainty" irrelevant pseudomosaicism might turn out to be a true mosaicism with clinical consequences after all.

#### P03.048

#### Azoospermia, cryptorchidism and dysmorphic features in a Tunisian male with a de novo apparently balanced reciprocal translocation t(4;6)(q34;q24)

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A de novo apparently balanced reciprocal translocation between the long arm of chromosome 4 and the long arm of chromosome 6; t(4;6)(q34;q24) is described in a Tunisian infertile man with azoospermia associated to an elevated FSH level and cryptorchidism. The patient is phenotypically abnormal and had bilateral external ear malformations with preauricular tags, asymmetric nose and abnormal extremities with bilateral thenar hypoplasia and Simian creases, triphalangeal thumbs and large toes.

No familial history of infertility, recurrent spontaneous abortions, hereditary disease or malformation is present. Furthermore no personal record of hearing loss or renal troubles is found.

Reciprocal translocation is defined as the exchange of chromosomal material between the arms of two heterologous chromosomes, thus changing the order, but usually not the amount of genetic material. But, in our patient dysmorphic features are remarkable and lead to expect cryptic unbalanced karyotype with or without involvement of others chromosomes like chromosome 16. Further characterization of this

translocation, by fluorescent in situ hybridization (FISH) using whole chromosome painting (WCP) probes as well as specific subtelomeric probes of the chromosome 4, chromosome 6 and others will be conducted. Auditory testing and renal ultrasound will be also considered for our patient as he shared some malformations with Townes-Brocks syndrome.

#### P03.049

#### Interspecies variation in recombination rate in mammals

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We analyzed frequency and distribution of recombination sites along meiotic chromosomes of the common shrew, mink, fox, cat and dog, using fluorescently-labeled antibodies to MLH1, a mismatch repair protein of mature recombination nodules, and compared our results with those obtained on mice and humans. The species studied differ in the total number of crossovers per cell and recombination rate. The common shrew has lowest recombination rate (0.38 cM/Mb). This is rather close to the estimates obtained by MLH1 mapping for male mice (0.44) and mink (0.48). Twice higher recombination rate is detected in men (0.83), dogs (0.76) and cats (0.72). The species with high recombination rate (man, cat and dog) have higher number of autosomal arms (FNa) than those with low recombination rate (mink, mouse and shrew). A comparison of the interference estimates coming from the distribution of MLH1 interfocus distances and RAD51/MLH1 focus ratio indicates a substantial difference between the species in the strength of interference. This factor contributes into interspecies difference in recombination rate. For example, cats have smaller FNa, smaller total length of synaptonemal complex, but weaker interference than dogs, and they exceed dogs in the average number of MLH1 foci. Selective changes of interference may serve as a mechanism of adaptive "fine adjustment" of recombination rate, additionally or alternatively to "coarse adjustment" achieved by fixation of the chromosome rearrangements that change FNa, such as pericentric inversions, tandem fusions and centromere shifts.

#### P03.050

#### Association between the gamma-aminobutyric acid type B receptor 1 (GABBR1) gene and schizophrenia

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Schizophrenia (SCZ), which affects approximately 1% of the general population, is a serious neuropsychiatric disorder that is characterized by diverse and variably expressed symptoms, which include disorganized thought pattern and severe neuropsychiatric disorder with a genetic component. The major inhibitory GABA-(gamma-aminobutyric acid) ergic system may be involved. The GABA type B receptor 1 (GABBR1) gene has been localized to 6p21.3, a region linked to SCZ. The present investigation five polymorphisms in the GABBR1 gene in a sample of DSM-IV SCZ probands and their families, 40 unrelated affected individuals matched with 40 healthy controls, using the proper questionnaire. Our research focused on the contribution of the GABBR1 gene variants to the risk for SCZ. This gene has been implicated in the etiology of several neurobehavioral disorders such as SCZ, juvenile myoclonic epilepsy, and dyslexia, and has been localized just distal to the HLA class I region. To our knowledge, this is the first attempt to study the variant in the GABBR1 gene in our regional SCZ. However, a weak significant difference was observed in the A-7265G polymorphism between the allelic frequency and a trend was observed between the genotype frequency of SCZ individuals and controls.

#### P03.051

#### Cytogenetic as a simple too for sex reversal study

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Advance in experimental endocrinology, biochemistry, genetics, and molecular biology have all contributed to our understanding of the process of human sex differentiation in the decades.

Based on the recognition Advance in experimental endocrinology, biochemistry, genetics Advance in experimental endocrinology, biochemistry, genetics, and molecular biology have all, and molecular biology have all of the underlying anomaly in the process of sexual differentiation

tion intersex disorders may be divided into abnormal gonadal determination and abnormal genital differentiation males with ambiguous genitalia but two differentiated testis are called MPH.

Females with ambiguous external genitalia but normal ovaries and normal internal genitalia are called FPH.

The XX males may be divided in to 3 subgroups:

46,XX males with the SRY gene(46,XX males without the SRY gene) and XX/XY mosaics.

DAX1 lies on the X chromosome. When it duplicates it causes an individual who is genetically male to develop physically as a female.

During the ten years (from 1997 to 2008) we have reported 46 patients referred for sex reversal abnormality which was more common in the female group (n=34, %0.7) compare to male population (n=12, %0.4). We conclude in here, that simple conventional cytogenetic methods are very helpful to identify male or female with sex reversal in addition molecular cytogenetic technology such as fish and molecular method to investigate the presence or absent of some critical genes such as SRY would be very useful to explain phenotype heterogeneity among sex reversal group male or female.

### P03.052

#### Automated sperm aneuploidy analysis

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Analysis of sperm aneuploidy level by fluorescence in situ hybridization (FISH) requires a large number of sperm to be evaluated. Automated analysis could increase the number of analyzed sperm and reduce the subjectivity.

We have developed a system using a motorized microscope (Zeiss Axioplan) with software for image acquisition and processing (Zeiss Axiovision) together with image analysis software (CellProfiler from Whitehead Institute for Biomedical Research, [www.cellprofiler.org](http://www.cellprofiler.org)) and our own application for reviewing and manual correction of results.

We have performed FISH with probes for chromosomes 18, X and Y on 12 semen samples. Frequencies of normal sperm bearing X or Y as well as frequencies of abnormal sperm with disomies and diploidies were evaluated by three means: manually, automatically and automatically with manual correction of abnormal results. The results were compared by the paired t-test.

Comparison of manual and automated analysis showed a significant difference in 6 out of 9 parameters: frequency of normal sperm with Y (P=0.004), disomy 18 (P=0.009), disomy XX (P=0.007), diploidy XX (P=0.013), diploidy YY (P=0.015) and diploidy XY (P=0.003). After manual review and correction none of the frequencies of abnormal results differed significantly from the results of manual evaluation.

Our preliminary results show that the automated sperm aneuploidy analysis has to be combined with manual correction of results.

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### P03.053

#### A retrospective study on the results obtained from cytogenetic analysis of couples encountering recurrent pregnancy loss in Iran

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Recurrent or habitual pregnancy loss (RPL) is a distressing problem affecting about 1% of all pregnancies. The condition is determined by loss of two or more pregnancies until weeks of 20<sup>th</sup> - 24<sup>th</sup> gestation. Balanced chromosomal rearrangements have been recognized as a major cause of recurrent miscarriages in women.

To evaluate the incidence of balanced chromosomal rearrangements in parents encountering RPL, we carried out a retrospective study on the results obtained from analysis of GTG-banded chromosomes prepared from lymphocyte culture of 400 couples, referred to our lab due to recurrent spontaneous abortions. Different types of chromosomal aberrations including robertsonian translocations (D:D, D:G and g:g), reciprocal translocations, inversions and mosaic form of chromosome X aneuploidy were identified in 12 percent of the patients.

The results indicate that the cytogenetic analysis of couples with two or more pregnancy losses can prevent the recurrence of chromosomal

aberrations at birth.

### P03.054

#### Evidence for significance of epigenetic inactivation of the cell-cycle checkpoints genes into etiology of chromosomal mosaicism during embryo development

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Chromosomal mosaicism is a feature of abnormal embryo development with increasing incidence during epigenetic genome reprogramming. Probably, aberrant DNA methylation may reinforce mitotic instability through inactivation of cell cycle checkpoints. However, relationships between mosaicism and epigenetic abnormalities are not investigated. The aim of the present research was delineation of impact of abnormal DNA methylation of G1/S-checkpoints genes into etiology of chromosomal mosaicism. Promoter methylation of *P14ARF*, *CDKN2B* and *RB1* genes was studied by methylation-specific PCR in the cytotrophoblast and extraembryonic mesoderm of 45 spontaneous abortions (SA) with chromosomal mosaicism, 20 SA with normal karyotype and 23 induced abortions. Level and tissue-specific distribution of aneuploid cells were estimated by interphase FISH. Abnormal methylation of *P14ARF* and *RB1* was observed in 9 and 20% of mosaics, respectively. At all 10 SA with epimutations were found. Low-level mosaicism confined by cytotrophoblast was denoted for 3 embryos indicating a post-zygotic origin of aneuploidy. At the same time epimutations were found in both tissues indicating an error of genome demethylation prior to implantation and germ layers differentiation. All other mosaics with abnormal methylation were developed from aneuploid zygotes. So, the epimutations were the secondary abnormalities for such embryos. However, in this group association between tissue-specific methylation and level of euploid cells was observed providing evidence for reinforcing of trisomy rescue under abnormal epigenotype. Moreover, the incidence of *RB1* methylation in the extraembryonic mesoderm (27.8%) was significantly higher than in the cytotrophoblast (5.7%, P=0.04) indicating a possible epigenetic mechanism of karyotype self-correction in the trisomic conceptions.

### P03.055

#### Detection of parental origin and cell stage errors of a chromosome X polysomy 49,XXXXY and new clinical findings

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Polysomy 49,XXXXY is a rare sex chromosome aneuploidy syndrome characterized by mental retardation, severe speech impairment, craniofacial abnormalities, multiple skeletal defects and genital abnormalities. There are very few reports concerning prenatal diagnosis of tetrasomy X, and the genetic mechanism by which it arises was not well investigated.

We describe a case with 49,XXXXY syndrome. The case had many characteristics of Fraccaro Syndrome; language impairment, mongoloid slant, epicanthal folds, peg shaped teeth, cryptorchidism, umbilical herni and dysmyelinisation findings. Conventional cytogenetic technique was applied for the karyotype analysis. The parental origin of polysomy X were identified by using Quantitative fluorescent polymerase chain reaction (QF-PCR) technique.

Cytogenetic analysis revealed a karyotype of 49,XXXXY for the case. QF-PCR with short tandem repeat (STR) markers specific for chromosome X revealed that all of the four X chromosomes were of maternal origin. These data provide additional evidence for the successive non-disjunctions in maternal meiosis I and II.

This kind of studies will improve knowledge about the mechanisms of aneuploidies, and enable appropriate genetic counseling.

### P03.056

#### A novel Reciprocal translocation t(11;22)

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A couple were referred for cytogenetic testing because they had a child with some form of pulmonary disorder. A standard karyotype was obtained and an abnormality reported as 46,XX,t(11;22)(q24;q12) found in the mother. This is a rare translocation.

## P03.057

**Pathological cytogenetic findings of chromosome 21 prenatally diagnosed in Medical Genetic Center of Novi Sad**

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We present results of prenatally detected trisomy of chromosome 21 in Medical Genetic Centre in Novi Sad which is a part of Institute for Children and Youth Health Care of Vojvodina. Prenatal genetic screening of fetal abnormalities is performed using detailed analyses of pedigree, maternal and paternal age, biochemical screening and expert ultrasound results such as thickness of nuchal translucency, absent nasal bone, hyperechogenic bowel, short femur, and other.

In this paper we present incidence of prenatally detected trisomies of chromosome 21 during last eight years (2000-2008) in Medical Genetic Centre in Novi Sad, Vojvodina northern part of Serbia with around 2.000.000 inhabitants. During last eight years we detected 113 trisomy of chromosome 21 (105 classical trisomies, 5 mosaical forms, 3 translocational forms), that is 38.96% of all prenatally detected chromosomal anomalies (N= 113/290; 38.96%).

## P03.058

**Dicentric Y chromosome in Turner syndrome**

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Present case relates to a 19 years old girl with Turner syndrome phenotype referred to our clinic for cytogenetic analysis. Patient presented primary amenorrhea and no signs of virilisation. Cytogenetic analysis revealed mosaic karyotype 46,X, dic (Y)(q11.2),45,X. FISH analysis confirmed presence of a dicentric Y chromosome. FISH analysis was performed using centromeric probe for X chromosome and specific probe for SRY region of Y chromosome.

Presence of Y chromosome material is associated with a ~12% risk of gonadoblastoma. Gonadoblastomas may transform into malignant germ cell neoplasm.

Conclusions: testing for Y chromosome material in Turner syndrome should be performed in any Turner syndrome patient.

## P03.059

**Parental Origin of X-Chromosome in Turner syndrome patients.**

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Turner syndrome (TS) is a chromosomal disorder in which all or part of one X chromosome is missing. Objectives: To detect the spectrum of chromosomal abnormalities; to determine the parental origin of the abnormality, and correlate it with the patient phenotype. The study included 42 females who had Turner stigmata (30 Egyptian; 12 Italian). The patients were classified into: Group (A) patients with only 45,X karyotype (n=16), group (B) patients having either mosaic cell lines or other X-chromosome abnormalities (n=26). Numerical X-chromosome mosaicism was observed in 22 patients (52%), 7 patients (17%) had an isochromosome X, 3 patients (7%) had a ring X chromosome, 1 patient (2%) had a deletion of the short arm of the X chromosome, 3 patients (7%) had an isochromosome X and 1 patient (2%) had a deletion of the long arm of the X chromosome. FISH technique was performed using Alpha satellite DNA cocktail probe for chromosome X and Y. A second cell line was detected in 4 patients who were diagnosed as having 45,X. We used PCR-based typing of highly polymorphic microsatellite markers distributed along the X chromosome to detect origin of the X chromosome. Parental origin of the single X chromosome was maternal in 67% of these patients, while in group (B), parental origin of the normal X chromosome was maternal in 11 patients (65%), paternal in 6 (33%), uninformative in 1 patient (6%). No evidence for X-imprinting of the studied physical features in TS was observed. Thus, there is no apparent clinical indication for investigation of X-chromosome parental origin in individuals with TS.

## P03.060

**XX male patient with Robertsonian translocation der(13;14)**

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Introduction: Commonly XX sex reversal is a result from translocation of Yp material including SRY gene onto the X chromosome.

Materials and Methods: We report a 27-year-old XX male with Robertsonian translocation. The proband was born to a 29-years woman from a second pregnancy. The first pregnancy has ended with childbirth of the healthy girl. The proband's sister is healthy, married, has the healthy daughter. The patient was referred to our centre with a 3 year history of male factor infertility. The proband had fully mature male genitalia with descended in the scrotum hypoplastic testes and no sign of undervirilization. His weight was 59 kg, height - 166 cm. Intelligence was normal. Semen analysis showed severe oligoasthenoteratozoospermia (sperm count 0.1 mln/ml).

Chromosome analysis was performed on cultured PHA-stimulated peripheral blood lymphocytes with GTG-, C- and QFH-staining in accordance with standard techniques. DNA was extracted from peripheral leukocytes by a standard method. Molecular analysis of Y chromosome loci was performed using multiplex PCR amplifications for SRY, AMELX/AMELY, ZFY/ZFX, and seven Yq-specific STSs: sY84, sY86, sY615, sY127, sY134, sY254 and sY255.

Results: Cytogenetic examination showed 45,XX,der(13;14)(q10;q10) karyotype in all of 50 analyzed metaphases. PCR amplifications were positive for SRY, AMELX, AMELY, ZFX, ZFY and negative for all analyzed Yq11 loci. The origin of Robertsonian translocation (de novo or inherited) was not found because of a material from the proband's parents and sister was not available.

Conclusion: To our knowledge we reported the first XX sex reversed patient associated with Robertsonian translocation.

## P03.061

**Wide spectrum of Peters and Axenfeld-Rieger anomalies secondary to a 4q25 microdeletion encompassing the PITX2 gene**

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Many genes are involved in the ocular development. The alterations of some of them are responsible of the Peters or the Axenfeld-Rieger anomalies (PITX2, FOXC1, PAX6 ...). In these conditions, angle anomalies are responsible of glaucoma in 50% of cases, usually congenital and difficult to manage. When they are associated with extra-ocular symptoms, the name of Rieger or Peter + syndrome can be used. In these entities, the mode of inheritance is usually autosomal dominant.

We report a 21-year-old patient, third child of healthy non-consanguineous parents with a negative familial history. He presented a Peters anomaly of the right eye requiring an iridectomy at 3 months of age, and an Axenfeld-Rieger anomaly of the left eye associated with a glaucoma at the age of 10. During childhood, he benefitted of several surgical interventions that concerned umbilical hernia, Meckel diverticulum, bifid uvula, posterior sub-mucous cleft palate, pharyngoplasty and tympanoplasty. He developed dysmorphic features including flat malar region and retrognathia requiring surgical repair at the age of 16. He also presented hypodontia and persistence of lacteal teeth. He had a normal mental development, a normal puberty and a normal stature (1m80), but secondary to his visual impairment, he studied in a special school.

Genetic investigations were negative for the standard karyotype and the sequencing of the genes PITX2, FOXC1 and PAX6. The array-CGH exhibited a de novo 1.7 Mb deletion at the locus 4q25 encompassing 13 genes including PITX2.

## P03.062

## Nine patients with a microdeletion 15q11.2 between breakpoints 1 and 2 of the Prader-Willi critical region: Is it clinically relevant?

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Behavioural differences have been described in patients with type I deletions (between breakpoints 1 and 3, (BP1-BP2)) or type II deletions (between breakpoints 2 and 3) of the 15q11.2 Prader-Willi/Angelman region. The larger type I deletions appear to coincide with more severe behavioural problems (autism, ADHD, obsessive-compulsive disorder). The non-imprinted chromosomal segment between breakpoints 1 and 2 involves four highly conserved genes, TUBGCP5, NIPA1, NIPA2, and CYFIP1; the latter three are widely expressed in the central nervous system, while TUBGCP5 is expressed in the subthalamic nuclei. These genes might explain the more severe behavioural problems seen in type I deletions.

We describe nine cases with a microdeletion at 15q11.2 between BP1-BP2, thus having an haploinsufficiency for TUBGCP5, NIPA1, NIPA2, and CYFIP1 without Prader-Willi/Angelman syndrome. The clinical significance of a pure BP1-BP2 microdeletion has been debated, however, our patients shared several clinical features, including delayed motor and speech development, dysmorphisms and behavioural problems (ADHD, autism, obsessive-compulsive behaviour). Although the deletion often appeared to be inherited from a normal or mildly affected parent, it was de novo in two cases and we did not find it in 350 healthy unrelated controls.

Our results suggest a pathogenic nature for the BP1-BP2 microdeletion and, although there obviously is an incomplete penetrance, they support the existence of a novel microdeletion syndrome in 15q11.2.

## P03.063

## A 17q21.31 deletion associated with progressive muscle hypertrophy and skeletal anomalies

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The 17q21.31 microdeletion is a recently described syndrome with characteristic features of developmental delay, hypotonia, long face, tubular or pear-shaped nose with bulbous nasal tip, and friendly behaviour. We report an 18 year old young man with cognitive delay who presented with hypotonia, bilateral radial head dislocation, laryngotracheomalacia, bilateral inguinal hernias, broad thumbs, large hands with fleshy fingers, seizure disorder, congenital partial fusion of lower thoracic and lumbar vertebrae, and deep hoarse voice. His facial features were strikingly similar to those previously reported in the 17q21.31 microdeletion syndrome and he had a very cheerful outgoing personality. In early childhood, he had marked ligamentous laxity and hypotonia, but his thigh muscles were prominent. After age 12, he began to develop prominent musculature in his upper extremities, without exercising. At age 18, he is quite strong and his muscle bulk surpasses that of his normal brother who is very physically fit. Microarray-based comparative genomic hybridization using a 44k oligo array revealed a deletion of approximately 627 kb in the 17q21.31 region (chr17: 41073486-41700815). The critical region for the 17q21.31 microdeletion syndrome consists of a 424 kb genomic segment (chr17: 41046729-41470954, hg17) encompassing at least 6 genes (C17orf69, CRHR1, IMP5, MAPT, STH, and KIAA1267). The proximal deletion breakpoint in our patient is distal to that seen in previously reported cases, possibly refining the critical region involved in the facial dysmorphic features and behaviour. Haploinsufficiency for a gene in a segment beyond the critical region may account for the muscle hypertrophy and/or skeletal anomalies.

## P03.064

## Clinical and molecular characterization of the 17q21.31 microdeletion syndrome in 11 French patients with mental retardation

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Array comparative genomic hybridization has recently led to the characterization of novel microdeletion and microduplication syndromes like the 17q21.31 microdeletion syndrome. Here we report the clinical and molecular characterization of 11 French patients with mental retardation and with the 17q21.31 microdeletion syndrome. We also present here the genotyping for H1/H2 and parent-of-origin analysis.

Several clinical features such as moderate mental retardation, childhood hypotonia, low birth weight and facial dysmorphisms (long face, tubular or pear-shaped nose and bulbous nasal tip) are common. Other inconstant clinically features include epilepsy, heart defects and kidney/urologic anomalies.

The described 17q21.31 critical region covers 424 kb and encompasses five reference genes (CRHR1, IMP5, MAPT, STH and KIAA1267). We report here a smaller ~ 200 kb deletion which encompasses only MAPT, STH and KIAA1267. This narrowing of the critical region point out the MAPT gene as candidate gene, especially since MAPT has been associated with several neurodegenerative disorders.

All these deletions arise de novo. A 900 kb inversion polymorphism exists in the 17q21.31 region and chromosomes with the inverted segment in different orientations represent two distinct haplotypes, H1 and H2. These different orientations are likely to facilitate the generation of the microdeletion through an established mechanism of NAHR and the offspring of carriers of the H2 lineage are predispose to deletion. In each trio tested, the parent-of-origin of the deleted chromosome 17 carries at least one H2 chromosome and, for informative cases, 2/3 out of deletions were of maternal origin and 1/3 of paternal origin.

## P03.065

## Chromosome 1q21.1 deletion and duplication in the patient with psychiatric problems

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Chromosome 1q21.1 deletions and duplications have recently been reported to show associations with developmental delay, schizophrenia and related psychoses, congenital heart defects, cataracts, micro and macrocephaly.

We report a rare case of chromosome 1q21.1 deletion and duplication in the same patient whose brother later presented with similar psychiatric symptoms and similar chromosomal rearrangement.

An 8 year old female with PDD NOS, ADHD, expressive language problems, developmental delay, mental retardation (IQ 60) and failure to thrive presented with severe aggressive behaviour that necessitated hospitalization. Her physical examination was remarkable for microcephaly (<3%), thin habitus (weight <3%, height 3%) and hyperextensible elbows. Her family history is significant for 2 sisters and 2 brothers all with hearing problems, a mentally challenged father, a maternal

grandmother and 2 maternal aunts with bipolar disorders. Biochemical and metabolic testing for inborn errors of metabolism and Fragile X DNA analysis were all normal. Chromosome microarray analysis revealed an XX female with 0.334 Mb duplication and a 0.984 Mb deletion at 1q21.1. Her brother with hearing problems who presented 2 months later with aggressive behaviour was shown to carry the same 0.334 Mb duplication and a 1.06 Mb deletion at 1q21.1. (Parental studies are not available yet).

Identification of 1q21.1 rearrangements should prompt the clinicians to evaluate the other members of the family and closely follow up the patients carrying these rearrangements. Given the spectrum of possible outcomes, there may be cases with subtle, undiagnosed findings (in our case, psychiatric problems in a brother) and early diagnosis might improve outcome.

#### P03.066

#### Cortical dysplasia and craniofacial dysmorphism in a child with interstitial microdeletion of 7q22.1-7q22.3 detected by array comparative genomic hybridization

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Array comparative genomic hybridization (aCGH) has proved useful as a diagnostic tool for patients with developmental delay, craniofacial dysmorphism, and normal karyotyping. Here we report the findings in a 4 year old boy who was referred to our center for evaluation of developmental delay and dysmorphic features. He was born at term with uneventful prenatal and neonatal periods. His parents, who have 4 other normal children, noticed that he had developmental delay since early infancy. His examination showed that he had macrocephaly, malformed right ear, retrognathia, hypotonia, and undescended testes. Brain MR imaging showed hypoplasia of the corpus callosum and thickening in the cortex of the frontal lobes predominantly in the singulate gyrus suggestive of cortical dysplasia. MR Spectroscopy was unremarkable. Karyotyping with 725 band resolution was normal. FISH for subtelomeric cryptic rearrangements / deletions / duplications was negative. Array CGH using a custom oligonucleotide microarray revealed an interstitial microdeletion of 7q22.1 to 7q22.3, spanning approximately 3.5 megabases. The deletion was confirmed by FISH study. To our knowledge, this particular microdeletion has not been reported in literature using aCGH. The phenotype we describe here in our patient may represent a unique genomic syndrome of 7q22.1-q22.3 microdeletion that could be clinically recognizable. Furthermore, our report supports the value of incorporating aCGH in the evaluation of patients with undiagnosed developmental delay and dysmorphism.

#### P03.067

#### Recurrence of a de novo 2q22.1q22.3 deletion identified by array-CGH in two sibling foetuses presenting precocious lymphoedema and associated malformations

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We report on two sibling foetuses presenting major lymphoedema during the first trimester of pregnancy, associated with several malformations. They were born from healthy and unrelated young parents. The first pregnancy was interrupted at 13 weeks of gestation because of the severity of the lymphoedema. Karyotype on chorionic villus sample was normal. Pathology revealed in a male foetus the association of a common mesentery, a unique ectopic testis and small renal cysts.

The second pregnancy was terminated at 15 weeks of gestation. The female foetus presented a generalized lymphoedema, a single umbilical artery and bilateral hydronephrosis.

Both foetus and parents had normal conventional chromosomal analysis. Array-CGH revealed a 6.7 Mb 2q22.1q22.3 deletion encompassing five genes in the two foetuses. Deleted genes in this region were *LRP1B*, *KYNU*, *ARHGAP15*, *GTDC1* and *ZEB2*. The latter is known to be involved in Mowat-Wilson syndrome.

No parental anomaly was detected by array-CGH. In addition, FISH analysis on both parents confirmed the de novo deletion. Recurrence

was explained by a presumed germline mosaicism.

Reported cases with identical structural chromosomal aberrations born to karyotypically normal parents are rare. Gonadal mosaicism is well documented for autosomal dominant and X-linked disorders, but seems to be very rare for this type of chromosomal anomaly. Array-CGH analysis can therefore be very helpful to identify small structural chromosomal aberration in foetuses with unusual multiple congenital anomalies syndromes, and for adequate genetic counselling.

#### P03.068

#### De novo 2q36.1-q36.2 including *PAX3* deletion in a patient with atypical Waardenburg syndrome type 1

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Waardenburg syndrome (WS) accounts for 2% of congenital deafness. It is a heterogeneous group of diseases divided into 4 subtypes. WS3 associates hearing impairment, abnormal pigmentation, dystopia canthorum and limb anomalies. This condition is due to mutations or deletions of *PAX3* gene. Mutations in *PAX3* have also been reported in the craniofacial-deafness-hand syndrome (CDHS), a rare form of WS, which comprises a flat midface, hypertelorism, a hypoplastic nose and limb anomalies.

Here we report on a four year-old boy referred to the genetic clinic for bilateral hand arthrogryposis. He also presented dysmorphic features such as a flat and asymmetrical face, severe dystopia canthorum and a small mouth. Neither depigmentation nor gastro-intestinal involvements were noted.

He was born at 38 WG with the following growth parameters: 1.650 kg (<3<sup>rd</sup> centile) for the weight, 45cm (10<sup>th</sup> centile) for the length and 30cm (3<sup>rd</sup> centile) for the HC. A brain ultrasound scan performed soon after birth was normal. On heart examination, patent ductus arteriosus, pulmonary hypertension and Wolf Parkinson White anomaly were identified. Subsequently he developed epilepsy and learning difficulties mainly in speech.

Array-CGH allowed the identification of a *de novo* 2q36.1q36.2 deletion which involves *PAX3*. Whole *PAX3* deletions have already been described in WS3, but our patient did not show all the classical WS signs, shared some common findings with CDHS but also presented some not previously described features in both conditions. This case allows broadening the WS spectrum but may also highlight *PAX3* deletions involvement in CDHS.

#### P03.069

#### Independent patients with an identical 9q31.1q31.3 deletion showing similar clinical features: a new microdeletion syndrome?

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Interstitial deletions of chromosome 9 long arm are rare and until now only 20 cases are described in medical literature. Moreover, most of them were detected by conventional cytogenetic techniques, whose limited resolution prevents a precise definition of the breakpoints and the assessment of accurate genotype-phenotype correlation.

We report two identical deletions of about 6.3 Mb, involving 9q31.1q31.3 region, found through array-CGH in two unrelated patients with common phenotypic features.

They both present mild psychomotor delay, gibbus dorsalis and peculiar craniofacial dysmorphisms, especially thick hair, pointed eyebrows, midface hypoplasia and mild prognathism, downturned bulbous tip of the nose and short neck. One of the two patients has a twin sister with highly similar dysmorphic features, which we are going to analyze for the presence of the above mentioned imbalance.

Comparing the genomic positions of all previously described deletions with those we detected, a common minimal region cannot be found because they are not all overlapping. However a better characterization of cytogenetically defined breakpoints and the finding of further

cryptic imbalances in the same chromosomal region, through the application of high resolution techniques, will lead to the identification of causative genes and underlying molecular mechanisms. The finding of identical deletions in unrelated patients suggests the occurrence of the same event mediated by peculiar sequences or structures. Indeed at the breakpoints, we found repetitive elements, such as Alu sequences, which are known to generate genomic instability and to promote chromosomal aberrations.

#### P03.070

#### Deletion 6p25.3-p24.3 and duplication 8q24.22-q24.3 in a patient with syndromic Axenfeld-Rieger malformation and mental retardation not detectable by standard karyotyping were identified by molecular karyotyping using Array-CGH analysis

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The terminal deletion of chromosome 6p (including *FOXC1*) leads to a well described and very characteristic phenotype with features of Axenfeld-Rieger syndrome (Axenfeld-Rieger eye malformations (AR), facial dysmorphism, teeth anomalies, and excessive periumbilical skin) plus mental retardation, heart defects, and deafness. The female patient we present here showed the full spectrum of these phenotypic features with a severe manifestation. However, no terminal 6p deletion was detected by standard karyotyping with a resolution of 400 - 550 bands. Therefore, Molecular Karyotyping by Array-CGH analysis was performed using the 36K OpArray V4 microarray (Operon, Cologne, Germany) and revealed a deletion of 6p25.3-p24.3 spanning 9.7Mb as well as an 8q24.2-q24.3 duplication of 15.0Mb. The complex structural rearrangement was finally confirmed by FISH-analysis and the initially suspected terminal deletion of chromosome 6p was found to be masked by a duplication of chromosome sub-bands 8q24.2-24.3.

#### P03.071

#### Fontaine syndrome is due to 7q21.3 microdeletion

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Fontaine syndrome was described in 1974 in a three generations pedigree. It is characterized by the association of mandibulofacial dysostosis, dysplastic ears, and split hand-split foot malformation. Other features have been described with a wide variability: cutaneous syndactylies of toes, cleft palate, epilepsy and mild mental retardation. By array-CGH analysis we found a 7q21.3 microdeletion in the previously reported patient from the original family and her affected daughter. This microdeletion ranging from 0.7 to 1 Mb contains 3 genes, namely *DLX5*, *DLX6* and *DSS1*, which play a role in limb embryogenesis. The two *DLX* genes are also implicated in inner ear development. To our knowledge, about twenty patients presenting larger chromosomal deletions involving this locus have been described in the literature, sharing common features with Fontaine syndrome: ectrodactyly of feet, microretrognathia and hearing loss or malformation of the inner ear. This syndrome was first linked to Patterson-Stevenson syndrome, described in one family in 1964, presenting the association of ectrodactyly, retrognathism, ear malformation and deafness. However, array-CGH analysis performed in this family was normal. Although Fontaine and Patterson-Stevenson are two syndromes sharing similar clinical features, these observations suggest a genetic heterogeneity or the possibility of deletions or mutations of a gene located in the deleted region.

#### P03.072

#### Identification of additional patients with interstitial deletions in the 12q14 interval highlights the role of *HMGA2* in regulating human growth

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Sciences, Mount Sinai School of Medicine, New York, NY, United States, <sup>4</sup>McGill University Health Centre, Montreal, QC, Canada, <sup>5</sup>Legacy Emanuel Children's Hospital, Portland, OR, United States, <sup>6</sup>Dept Medical Genetics, University of British Columbia, Vancouver, BC, Canada, <sup>7</sup>Center for Medical Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands. The 12q14 microdeletion syndrome is a rare but distinct disorder characterized by learning disabilities, short stature and Buschke-Ollendorff lesions in bone and skin. We have now identified 5 unrelated patients with this microdeletion syndrome using genome wide array CGH analysis. All patients have mild mental retardation and growth failure with low birth weight at term (on average 2240 g), failure to thrive in infancy and short stature (- 2.7 to - 6.2 sd) in childhood. Osteopoikilosis lesions are observed in 3 patients and skin lesions reminiscent of the Buschke-Ollendorff syndrome are present in 2 cases. Renal anomalies are found in 2 patients. The deletions are variable in size, ranging from 3.44 to 8.95 Mb. The breakpoints are non-recurrent and not flanked by segmental duplications. The 5 microdeletions overlap and define a critical interval of 2.61 Mb on chromosome band 12q14.3 encompassing 10 RefSeq genes, including *LEMD3* and *HMGA2*. We have shown previously that haploinsufficiency for *LEMD3* results in the Buschke-Ollendorff syndrome. Transgenic mouse models and association studies have suggested that *HMGA2* is regulating human growth. To further prove this hypothesis, we have investigated the 2.61 Mb critical interval in a series of patients with growth failure. In a mother and son with proportionate short stature (height at - 2 sd) we could show the heterozygous presence of an intragenic deletion encompassing exon 3 of the *HMGA2* gene. This observation confirms the role of *HMGA2* in human growth regulation and opens new diagnostic perspectives in children with so-called idiopathic short stature.

#### P03.073

#### Interstitial deletion 3p14 associated with multiple malformations and mental retardation - case report.

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Deletions of chromosome 3 usually involve the terminal segment 3p25-3pter in the well known 3p deletion syndrome. Interstitial deletions of the proximal short arm of chromosome 3 occurring as constitutional aberrations are rare and a defined clinical phenotype is not established, yet. In this paper we report the case of a 5 years-old boy with dysmorphic facial features (including broad forehead, short palpebral fissures, epicanthic folds, broad nasal bridge, short philtrum, small malformed ears, micrognathia), clinodactyly of 5<sup>th</sup> finger, severe psychomotor retardation, speech delay, congenital heart malformation, deafness and genital malformations (small penis, small scrotum, cryptorchidism).

Cytogenetic investigations have been performed on slides obtained from peripheral blood cultures by standard procedures. The karyotype established on high resolution GTG banding spreads exhibited an interstitial deletion of the proximal short arm of chromosome 3, del(3p14).

Clinical manifestations of this child will be compared with those of other patients with the same deletion previously described to further delineate the proximal 3p deletion syndrome, which seems to be quite different both of clinical and cytogenetic point of view from 3p25-3pter deletion syndrome.

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#### P03.074

#### High Resolution Agilent 244K oligoarray CGH analysis in patients with MR/MCA/DD

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Mental Retardation (MR) and Developmental delay (DD) with or without multiple congenital anomalies (MCA) occur in 2-3% of the general population and are very heterogeneous entities. Clinical characteristics of these patients are not always related to specific syndromes. Array comparative genomic hybridisation (aCGH) is a high-throughput

method used to detect small copy number changes within the genome that are not always visible by conventional karyotyping (>5-10Mb). Thirty patients with various degrees of MR, seizures, dysmorphic features and/or single or multiple congenital abnormalities and normal previous conventional karyotype, many of which had also received a variety of other genetic tests (FRAX, RETT, single FISH tests or metabolic screens), were analyzed with Agilent 244K oligoarrays, allowing a theoretical resolution of >50Kb. Clinically significant submicroscopic imbalances were detected in 15 (50%) patients. We are currently in the process of confirming our findings with QPCR. The high percentage of positive patients is probably due to the strict criteria of patient selection. The clinically relevant results are presented in the table below. Array CGH is a powerful tool for the identification of novel chromosomal syndromes and for more accurate prognosis and phenotype-genotype correlations.

ID	Chromosome	Gain/ Loss	Position start (UCSC hg18)	Position End (UCSC hg18)	Length (Mb)	Known syndrome	Genes*
1	1p36.33-p36.32	Loss	554,268	3,332,601	2.8	Yes	PLCH2, SKI, GABRD
		Gain	220,916,807	221,288,347	0.371	No	AIDA, DISP1
2	Xq11.3	Loss	44,005,210	44,048,137	0.043	No	EFHC2
		Loss	90,917,565	91,035,630	0.118	No	PCDHX
3	2q35	Loss	216,886,022	217,664,090	0.68	Yes	SMARCAL1
		Loss	70,598,263	71,795,160	1.2	No	FOXP1
4	5p15.33	Loss	948,032	2,209,449	1.26	No	TERT, SLC6A3, SLC6A19
		Loss	133,644,221	135,084,833	1.44	Yes	NKX6, CALY
5	3p14.1	Loss	70,951,444	71,601,477	0.65	No	FOXP1
		Loss	1,515,185	2,499,331	0.984	No	DUSP8, TNNI2, TGF2, TH, ASCL2, KCNQ1
6	11p11.2	Loss	43,833,775	44,553,392	0.719	Yes	EXT2, ALX4
		Loss	134,182,454	135,356,716	1.17	Yes	NKX6, CALY
7	17q21.31-q21.32	Loss	41,331,503	42,142,422	0.810	Yes	MAPT, STH, NSF
		Loss	41,395,772	41,983,466	0.587	Yes	MAPT, STH
8	17q21.31-q21.32	Loss	33,121,179	33,204,123	1.09	Yes	HNF1B, LHX1, DUSP14
		Loss	43,208,140	43,765,770	0.557	No	MAOB, MAOA, NDP
11	15q11.2	Gain	18,454,050	20,249,945	1.79	Yes?	
		Loss	21,982,746	22,396,107	0.413	Yes?	
12	15q11.2-q14	Gain	19,109,124	36,837,570	17.73	Yes	AS/PWS
		Loss	124,232,611	135,251,538	11.0	No	
14	18p11.32-p11.21	Loss	4,316	15,370,683	15.3	No	TGF1, USP14, NDC80, ADCYAP1
		Loss	62,447	7,455,153	7.39	Yes	WHS*

\*clinically relevant

### P03.075

#### *De novo* 1.2 Mb deletion in 2p16.3, disrupting the *NRXN1* gene in a boy with autism and developmental delay

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Autism spectrum disorders (ASD) are a group of neurodevelopmental conditions characterized by deficiencies in behavior, communication and socialization. Recent research has shown that different genetic factors are implicated in the aetiopathogenesis of ASD, including point mutations of several genes (such as *NRXN1*, *NLGN3*, *NLGN4*) as well as copy number variants on many chromosomes.

We describe a 6-year-old male with a 1.2 Mb *de novo* deletion of 2p16.3 detected by aCGH (Agilent 244K). Most of *NRXN1* is missing and no other known genes are within the deletion. The child has minor dysmorphic features: triangular open mouth, inverted and supernumerary nipples. Growth parameters, including head circumference are within normal limits. At the age of 9 months he had several seizure episodes successfully controlled with valproic acid. Mental development is delayed (absent speech) and the patient presents typical autistic features. Brain MRI was unremarkable.

As the deletion is *de novo* and the missing segment only encompasses part of *NRXN1*, it is highly likely that haploinsufficiency of this gene is causally related to the patient's phenotype. This finding will hopefully shed more light in the *NRXN1* function, deletions of which have additionally been associated with susceptibility to schizophrenia.

### P03.076

#### Molecular cytogenetic analysis of a boy with proximal 3q deletion syndrome

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Interstitial deletions of proximal 3q are rare. To our knowledge 11 cases have been described in literature, and just one of them was analysed at the molecular level. The patients show distinct and recognisable facial dysmorphism with prominent forehead, epicanthal folds, flat and broad nasal root, and anteverted nares. Structural brain, genitourinary and musculoskeletal abnormalities are also observed in the majority of cases. The severity of the phenotype usually correlates with the deletion size, but a clear genotype-phenotype correlation has not been established yet.

We present a male patient with prenatally detected hydrops. Karyotyping of umbilical cord blood showed a large deletion of the proximal 3q (karyotype 46,XY,del(3)(q13.2q21)). Caesarean section in the 34th week of gestation was performed due to foetal distress. Intensive care with ventilation support was necessary to combat the apnoea. The newborn had depressed nasal bridge, hypertelorism, low set ears, short neck, thick arms, single palmar crease on the right hand, hepatomegaly, and hypoplastic penis. Head ultrasound detected agenesis of corpus callosum. Microarray CGH analysis showed a deletion between Mb 108.3 and 129.0 of chromosome 3 affecting about 130 protein-coding genes. When compared to other published cases, the extent of the deletion in our patient is rather large, and this is in accord with his relatively severe phenotype. The detailed analysis of deletion overlaps and their gene content allows a speculation about the role of individual genes in the symptoms of the syndrome. Supported by grants IGA NR/9457-3 and MZO00064203.

### P03.077

#### A new case of proximal interstitial deletion of 6q analysed using array CGH

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Interstitial 6q deletions are relatively rare. About 60 cases have been described in literature. Three different phenotypic groups according to the localisation of the deletion have been suggested: proximal (6q11-q16), characterised by upslanted fissures, thin lips and hernias; middle (6q15-q25), with microcephaly, hypertelorism, intrauterine growth retardation, respiratory problems and limb malformations; and distal (6q25-qter), with cleft palate, retinal abnormalities, genital hypoplasia, and seizures. Hypotonia, ear anomalies, facial dysmorphism and mental retardation are common to all three groups. The localisation of the distal breakpoint is more important for the phenotype than the proximal one.

We present a girl with mental retardation, hypotonia, facial dysmorphism (high forehead, hypertelorism, epicanthal folds, dysplastic ears), single palmar crease on the right hand, pectus excavatum, delayed myelinisation, and bilateral frontal lobe atrophy. Her karyotype was 46,XX,del(6)(q13q15). The phenotype was in agreement with that of the proximal 6q deletions. mbAND analysis and high resolution array CGH showed that the deletion was more centromeric, involving the 6q11-q14.1 region. The length of the deletion was about 15 Mb and it contained about 45 protein-coding genes. The distal breakpoint mapped to intron 1 of the *MYO6* gene. The proximal breakpoint was in the centromeric genome assembly gap. At present we are attempting to obtain the nucleotide sequence of both breakpoints. To our knowledge, only 19 patients with proximal 6q deletions have been described, and none of them was analysed using array CGH. The deletion in our patient is unique. Supported by grants MZO00064203, MZOVN2005, INCORE and CHERISH.

### P03.078

#### Further delineation of the phenotype of the 21q22.11q22.12 deletion encompassing the *RUNX1* gene

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Familial thrombocytopenia (OMIM 601399) is a rare inherited disorder due to mutations in the *RUNX1* gene, localized in 21q22.12. Affected individuals have mild to moderate thrombocytopenia and are prone to develop myelodysplasia and acute myeloid leukaemia in 1/3 of cases. Deletions including the *RUNX1* gene have been reported in patients presenting with syndromic thrombocytopenia. We report two new cases, a 23 year-old boy and a 7 year old girl. Both developed mild asymptomatic thrombocytopenia. Psychomotor development was severely delayed and both developed severe epilepsy and behavioral problems with agitation and restlessness. On examination the two patients had dysmorphic features, with microcephaly, protruding and everted lips, down turning corners of the mouth and bulbous nasal tip. Hypoplastic nipples, short fingers with broad proximal interphalangeal joints, camptodactyly, broad halluces, toenail hypoplasia and complete agenesis of corpus callosum were noted in the male patient. Deletions at 21q22.11q22.12 including *RUNX1* were detected by array-CGH in the two cases. The two deletions overlapped with distinct breakpoint. Recently, genomic deletions overlapping the 21q22.11q22.12 region were reported in 5 patients. All had thrombocytopenia, growth retardation and mental retardation and one developed AML. The facial appearance of the three patients with published pictures is very similar to the one of our patients. Interestingly, the size of the deleted region is variable and breakpoints are specific to each patient, suggesting a mechanism different from the classical non-allelic reciprocal recombination. The minimal overlap region contains 13 genes, some of which are expressed in the central nervous system.

#### P03.079

#### Duplication of the GPC3/GPC4 gene cluster on Xq26.2 detected by Array-CGH in a family with developmental delay/mental retardation and dysmorphic features. A new syndrome ?

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Simpson-Golabi-Behmel (SGBS) syndrome is an X-linked overgrowth syndrome characterized by pre- and postnatal overgrowth, a characteristic facial appearance and different congenital malformations. SGBS is caused by mutations or deletions of the glycan 3 (GPC3) gene. Here, we report on a 2-year-old boy presented with psychomotoric retardation, growth retardation, microcephaly, mild congenital malformations (micropenis, hypospadias) and dysmorphic features (e.g. broad forehead, facial asymmetry, round face, hypertelorism, micrognathia, deep set and posteriorly rotated ears, slight bilateral clinodactyly). Initially, Silver-Russell-syndrome was suspected. While karyotyping and testing for Silver-Russell syndrome were negative, a 1-4 Mb duplication at Xq26.2 including the GPC3/GPC4 gene cluster was identified by BAC array-CGH. This finding was validated by MLPA and Q-PCR analysis.

For the characterization of the duplication in a much higher resolution we have designed and customized a 60mer oligo array printed in a 105K format (105.000 oligonucleotides probes) using the eArray technology (Agilent).

Testing of more family members revealed that the mother, the grandmother and a maternal uncle were carrier of the Xq26.2 duplication. All carriers displayed the characteristic features of the syndrome to some extent.

Interestingly, comparison of the Xq26.2 duplication phenotype to the phenotype of SGBS patients revealed partially a "reverse" phenotype to the SGBS.

Recently, it was proposed that the SGBS phenotype is caused by a misregulation of the hedgehog signal transduction pathway. Here we will present a potential explanation of the phenotypical differences between the two syndromes based on the function of glycan 3 in the hedgehog signal transduction pathway.

#### P03.080

#### De novo cryptic deletion at 2q14 in two female patients with Turner syndrome stigmata

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**Case1.** 13 years old, weight 37 Kg (3th-10th centile), height 133 cm (<<3th centile), OFC 53,1 cm (50th centile). She presented mild mental retardation, short stature, triangular face with bitemporal narrowing, "webbed" neck and low posterior hairline, drooping eyelids, thin nose, high palate, thin lips, shield chest, mild cubitus valgus, multiple pigmented nevi, atrial septal defect, hypothyroidism. Menarche occurred at 15 years of age after hormonal stimulation. Molecular analysis of *SHOX* gene resulted to be normal. Karyotype on blood and fibroblasts excluded the presence of a 45,X cell line.

**Case2.** 14 years old, weight Kg 45 (25th centile), height 154 cm (10th-25th centile; expected according to midparents' stature: 167±7 cm), OFC 53,1 cm (3th-10th centile). She presented moderate mental retardation, low posterior hairline, high nasal bridge, thin and convex profile of nose, short philtrum, thin lips, microstomia and prognathism, small and simple ears, clinodactyly, cubitus valgus, multiple pigmented nevi. Menarche occurred spontaneously at the age of 13+8/12 years. Karyotype: 46,XX,t(2;8)(q21;q24.3).

Array-CGH (Agilent platform, 244 k) allowed to identify 2q de novo interstitial deletions of 8 Mb in case 1 (proximal breakpoint at 113,120 Mb; distal at 122,079 Mb) and of 14 Mb (proximal breakpoint at 118,332 Mb; distal at 132,366 Mb) in case 2, with an overlapping region of about 3,7 Mb.

Conclusion: a critical deletion region at 2q14 (from 118,332-122,079 Mb, assembly March 2006) is associated with postnatal short stature, low posterior hairline, shield chest, cubitus valgus, multiple pigmented nevi, reminiscent of TS.

#### P03.081

#### Overlapping deletions in 10q22: Characterisation of a novel genomic disorder and identification of C10orf11 as a candidate gene for mental retardation

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Interstitial deletions of chromosome band 10q22 are rare, and very little is known about the mechanisms underlying their formation and about the contribution of individual genes to the clinical problems. We characterised interstitial 10q22 deletions in 3 unrelated mentally retarded patients by high-resolution array CGH, and we performed breakpoint analysis by array painting in a mentally retarded patient with a balanced chromosome translocation 46,XY,t(10;13)(q22;p13)dn. Patient 1 had a 7.9 Mb deletion in 10q21.3-q22.2. Patients 2 and 3 had nearly identical smaller deletions of approximately 3.4 Mb. The centromeric breakpoints of these two deletions were near an identical low-copy repeat (LCR), but there were no LCRs at the distal breakpoints which differed by 250 kb. Breakpoint analysis in patient 4 revealed the disruption of C10orf11, a brain-expressed gene that is located in the common deleted interval of patients 1-3. The presence of an LCR at the centromeric breakpoints of patients 2 and 3 suggests a common, LCR-mediated mechanism for the formation of both deletions. Since there were no corresponding LCRs at the telomeric breakpoints, this mechanism is probably different from non-allelic homologous recombination. The disruption of C10orf11 in a similarly affected patient suggests that haploinsufficiency of this gene is a major causative factor for mental retardation in del(10)(q22) patients.

## P03.082

**High-resolution array analysis detects microdeletions of chromosome region 16q22.1 in two unrelated individuals with MR/ MCA syndrome**

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Here we report a detailed clinical and molecular investigation of two unrelated individuals, an 18-months-old boy and a 13-years-old girl with de novo overlapping deletions of 16q22.1, sized 1.48Mb and 0.28Mb, respectively. Clinically they both presented with dysmorphic features, moderate developmental delay, sleeping disturbances, and abnormal behaviour. The boy had low birth weight, short stature (-4SD), microcephaly (-4SD), high forehead, thin eyebrows with "medial flaring", strabismus, narrow flat nasal bridge, prominent columella, short flat philtrum, thin lips, late erupted dysplastic teeth, dysplastic ears, short fingers and toes, broad 1<sup>st</sup> toe, delayed skeletal age. He showed distinct externalising behaviour, friendly and continuously smiling. The girl had autistic behaviour, hypermetropia (+6), no signs of puberty at the age of 13, and dysmorphism such as hypertelorism, thick eyebrows, long eyelashes, epicanthic folds, wide pronounced nasal bridge, low set posteriorly rotated dysplastic ears, long prominent philtrum, thin lips, hairy skin, sandal gap, broad 1<sup>st</sup> toe. Chromosome aberrations were detected by high-resolution array analysis, 250k SNPs array (Affymetrix) for the 1.48Mb deletion and 244k CGH array (Agilent) for the 0.28Mb deletion. Breakpoints on chromosome 16 were defined as 65101151-66582496 (hg18) and 65958487-66235411 (hg18), respectively. Several possibly relevant genes are located in the overlapping gene-rich region such as *CTCF*, *ZDHHC1*, *TPPP3*, *LRRC36*, and *AGRP*. Authors discuss reasons for partially overlapping/partially distinct phenotypes of the two individuals. To our knowledge these are the first two cases reported with microdeletions confined to the 16q22.1 region.

## P03.083

**A de novo 305 kb interstitial dup(3)(p25.3) encompassing the VHL and *IRAK2* genes in a patient with mental retardation/multiple congenital anomalies, epilepsy, spasticity and ectomorphic habitus**

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Partial duplications of the short arm of chromosome 3 are very rare. Often they are reported in translocations involving other chromosomes, whereas deletions encompassing the *VHL* gene in 3p25.3 predispose to Van-Hippel Lindau syndrome.

While screening for genomic copy number variations (CNV) with a 1 Mb resolution bacterial artificial chromosome (BAC) array-based comparative genomic hybridisation (aCGH) in a 17 year-old male referred for the aetiological diagnosis of mental retardation and multiple congenital anomalies (MR/MCA) and ectomorphic habitus, a *de novo* 3p25.3 microduplication was detected and refined by Multiplex Ligation-dependent Probe Amplification (MLPA) to a 305 kb region encompassing the *VHL* and *IRAK2* genes and disrupting the *GHRL* gene. This is not reported as a benign CNV in the database of genomic variants (DGV). Moreover, we have not found similar duplication in the DNA of parents of nearly 1000 patients we tested by aCGH.

The patient was followed since the age of six years. He has no tumour and there is no history of familial cancer. Brain MRI was normal.

Interestingly, duplication of *IRAK2* can cause epilepsy by activating the *NFKB* signalling pathway, both controlling the postsynaptic glutamate receptor density. Disruption of the *GHRL* gene can explain the ectomorphic habitus described here, since patients with larger dup(3p) involving *GHRL* show short stature and obesity.

To our knowledge, this is the smallest duplication of chromosome 3p encompassing the *VHL* region reported yet. The prognosis of this cytogenetic imbalance is unknown and a long-term follow-up is essential for an early diagnosis of malignancy.

## P03.084

**Array-CGH for the identification of constitutional copy number changes**

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Up to date, we have analyzed 331 patients with a conspicuous phenotype e.g. dysmorphic features, mental retardation, and developmental delay by array-CGH. We employed different array platforms, including the 1 Mb or 8k large insert clone arrays and the commercially available 44K and 244K oligoarrays from Agilent.

We identified copy number changes (CNCs) in 79 patients (=23.8%). Subsequent analyses usually include evaluation of the parental genomes and verification of the array-CGH results either by FISH, MLPA or qRT-PCR.

We identified well-known microdeletion/duplication syndromes like Prader-Willi-, Smith-Magenis-, Di-George-, Miller-Dieker-, Williams-Beuren-, Phelan-McDermid-, Cri-du Chat-, Wolf-Hirschhorn-, or the 2q37 microdeletion syndrome. In addition to the detection of these well known syndromes array technologies paved the way for the detection of new deletion/duplication syndromes. The recently described deletions/duplications of 1q21.1, 8p23.1, 15q24, 16p13.11, 16p11.2 were also detected in our patients demonstrating the great clinical implication of these array-CGH findings. However, most of the detected CNCs are private. To achieve better genotype/phenotype correlations and to identify new clinically relevant syndromes, clinical and genetic data from all consented patients are entered into the DECIPHER database and are compared with the other entries in the database.

An especial interesting case represented a *de novo* deletion of 770 kb on chromosome 17p13.1, harboring the tumor suppressor gene *p53*, which was identified in a girl with developmental delay. Furthermore, we found that in this patient the breakpoint disrupted the transcriptional control of the *GUCY2D* gene, which likely causes the amaurosis of the patient.

## P03.085

**Interstitial *de novo* del(1)(q25.1q31.3): clinical presentation and molecular description with array-CGH**

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We report a 11-year-old male child with prenatal onset of growth retardation, growth hormone deficiency, developmental delay and several dysmorphic features including significant orthodontic problems, severe malocclusion, protrusion of upper jaw and narrow high-arched palate with triangular shaped palate, low hairline posteriorly, hypertelorism, long palpebral fissures, mild epicanthal folds bilaterally, long eyelashes and reversion of lower eyelid, relatively prominent ears, small hands and digits with 5th finger clinodactily bilaterally and apparently persistent finger pads, small feet and toes with right 2nd-3rd toe syndactyly and increased gap between 1st and 2nd toes bilaterally and Achille's tendon contractures bilaterally. He also has severe myopia, strabismus and mild astigmatism.

Molecular karyotyping using a 1 Mb resolution BAC array demonstrated the presence of a 21 Mb sized deletion on the long arm of chromosome 1. The deletion flanking clones are RP11-552K17 and RP3-433G19. The karyotype is arr cgh 1q25.1q31.3 (RP5-1045J21->RP11-435N12)x1. The case is compared with similar cases from the literature of postnatally detected interstitial deletion on 1q. This is the first time that array-CGH analysis is used for a more accurate assessment of the breakpoints in a patient carrying a deletion in the 1q25-q31 region.

## P03.086

**Array-CGH in fetuses with polymalformations**

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The importance of array-based comparative genomic hybridization (aCGH) for detecting unbalanced genomic aberrations including microdeletions, duplications and subtelomeric rearrangements have been largely demonstrated in postnatal series. We analyzed a series of 25 fetuses using aCGH (IntegraChip) with at least three malformations and normal karyotype. Fetal DNA was extracted from frozen sample and analyzed with 1-Mb BAC-arrays, including 4898 clones with a resolution of 600 kilobases. A genomic disorder was found in 2/25 fetus (8%), both confirmed by FISH analysis. The first case was a fetus with a de novo 15q26qter deletion (7.2Mb) associated with a phenotype compatible with Fryns syndrome: congenital diaphragmatic hernia (CDH), dysmorphic features, cardiac abnormality (aortic stenosis and hypoplasia left cavities) and hypoplastic distal phalanges. The deletion overlaps the critical region of CDH in 15q26.1q26.2. The second fetus presented de novo 13q32.2qter deletion (14Mb) associated with growth retardation, craniofacial dysmorphism, foot anomalies (clubfeet) and hypoplastic kidneys. This study demonstrates the utility of the array-CGH technology in detecting chromosomal abnormality in fetuses, allowing genetic counselling for the family. Observation 1 can also be of interest since it could be a clue in the search for the genes responsible for Fryns syndrome. The detection rate in our series is comparable with the two studies of fetuses with malformations analysed with BACs-array 300 and 3500 clones (8% and 7.3 % after exclusion of inherited anomalies) [Le Caignec et al., 2005; Schaeffer et al., 2004].

#### P03.087

##### Detection of known microdeletion syndromes on array-CGH: when the boundaries of clinical diagnoses are reached.

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The majority of recognizable syndromes had already been well described in their typical clinical presentations before classical recurring microdeletions were identified. The limited resolution of the conventional cytogenetics still fails to detect submicroscopic chromosomal imbalances. Therefore, suggestive phenotypes lead to targeted Fluorescence Hybridization In Situ (FISH) that is the most appropriate approach to confirm the diagnosis. Conversely, an apparently "chromosomal" but not specific developmental disorder with normal conventional karyotype can benefit from a new comprehensive high-resolution approach: Array-Comparative Genomic Hybridization (Array-CGH). It can however be very disappointing for the clinician when a known microdeletion syndrome is diagnosed by this latter technique.

In our experience, array-CGH identified ~15% chromosomal imbalances in about one thousand individuals presenting unexplained developmental disorders. Nine of them (0.9%) presented a known microdeletion syndrome not diagnosed on clinical findings. Three were terminal deletions (two 1pter and one 22qter) and 6 were typical interstitial deletions (3 Di-George, 1 Smith-Magenis, 1 Angelman and 1 Williams-Beuren syndromes).

We present some representative examples and propose hypotheses to explain the misdiagnoses:

- The phenotype can be atypical in particular for syndromes with important clinical variability such as 22q11.2 deletion
- The analysis of the dysmorphic features may be difficult when the patient is adult
- Some recently identified syndromes such as 22qter deletion may be "missed" by some clinicians
- The phenotype can be suggestive but associated with rare or none previously reported features.

#### P03.088

##### Whole-genome array-CGH screening in undiagnosed syndromic patients: old syndromes revisited.

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Most microdeletional syndromes were presumed to be well defined clinical entities. However, the introduction of whole-genome screening led not only to the description of new syndromes, but also to the recognition of a broader spectrum of features for well-known syndromes. Here we report on 3 patients presenting with mental retardation and normal standard karyotype. Patient 1 was a 37 year-old male with profound mental retardation, severe psychiatric disturbances within the autism spectrum, non-specific dysmorphic features and bilateral retinal detachment. 105K array-CGH Agilent permitted to diagnose a 1.6 Mb 17p11.2 microdeletion including the *RA1* gene, smaller than the common microdeletion usually found in Smith-Magenis syndrome. Patient 2 was a 31-year-old female with mild mental retardation, severe anxiety, non-specific dysmorphic features and short stature with micro-melia (142 cm) and X-rays compatible with hypochondroplasia. 105K array-CGH Agilent revealed a 1.4 Mb 7q11.23 microdeletion, similar to the deletion found in Williams syndrome. Patient 3 was a 6-year-old boy with mild mental retardation, non-specific facial dysmorphism and autistic features. BAC-array (Integragen, 1Mb) also revealed a 7q11.23 microdeletion including the *ELN* gene. Reevaluation in the 3 patients confirmed that the diagnosis was not possible on clinical grounds and emphasize that well-known genomic disorders can be phenotypically heterogeneous and more variable than originally thought. The large use of array-CGH might lead that such patients may be more readily achieved on the basis of genotype rather than phenotype.

#### P03.089

##### 44K array-CGH in 1000 patients presenting mental retardation/multiple congenital malformations.

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Since 2008, 11 array-CGH platforms have been enforced in France in order to detect constitutional cryptic genomic imbalances as a routine diagnosis.

To date, 1000 DNAs from patients presenting with MR/MCA have been studied using Agilent 44K array-CGH in our centre: 914 post-natal cases and 86 prenatal cases (63 after termination of pregnancy and 13 during pregnancy).

Considering post-natal cases, 160 (17.5%) genomic imbalances have been detected: 128 (14%) were considered deleterious (i.e. de novo or transmitted from a parent showing the same phenotype), 13 (1.4%) were found in a healthy parent but for the remaining 19 (2.1%) no parental samples were available. For the prenatal cases, 15 (17.4%) genomic imbalances were detected: 12 (14%) were considered deleterious and 3 (3.5%) were identified in a healthy parent.

Among the 140 deleterious genomic imbalances:

- 11 (7.9%) corresponded to known microdeletion syndrome not diagnosed on clinical findings: 3 were telomeric (two 1pter and one 22qter) and 6 were typical interstitial (three del 22q11.2 (DiGeorge), one del 17p11.2 (SMS), one del 15q11.2 (Angelman syndrome), one 7q11.23 (Williams-Beuren)). Two were Xq28 duplications involving *MECP2*.
- 4 (2.9%) were imbalanced telomeric anomalies (partial monosomy associated with partial trisomy).
- 23 patients presented new microdeletion syndromes. Three del 17q21 (*MAPT*), 3 del 15q13.3 (*CHRNA7*), 6 del 1q21/3 dup 1q21 (microcephaly/macrocephaly), and 4 del 16p11.2/7 dup 16p11.2.
- 5 patients presented single gene deletions.

All detected anomalies are available on the BACH database (<https://www.genopole-lille.fr/bach/menu.php>) with login/password on demand.

### P03.090

#### Creating a First-Generation Chromosome 18 Gene Dosage Map

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**Background.** Microarray technology has revolutionized the field of clinical genetics. For individuals with chromosome abnormalities, the promise of microarray technology is that it can quickly identify which genes are and are not present in two copies. In order for this information to be useful to clinicians, there must be some tool to link genotypic data with annotated phenotypic information. As an initial step towards this goal, we have created a first generation gene dosage map for chromosome 18. **Methods.** Data from OMIM, the Database of Genomic Variants, and medical and scientific manuscripts were reviewed for each of the genes shown on the UCSC Genome Browser using the March 2006 assembly. 253 genes were classified as haplosufficient, haploinsufficient, conditional haplosufficient, or haplolethal. This information, along with our data on critical regions for 18q- phenotypic features, was used to create a custom track on the UCSC genome browser. **Results and Discussion.** Eighty-one genes were determined to be haplosufficient; 4 were haploinsufficient, and 1 was conditional haploinsufficient. The effects of the non-haploid state in the remaining genes were unknown. Critical regions for aural atresia, renal abnormalities, growth hormone deficiency, and dysmyelination were also included on the custom track. This map allows clinicians to align the molecular karyotype information from an individual patient with the annotated genomic content so as to provide a clinical prognosis. Thus, we have taken the first step towards creating a genomic map that may be used in counseling and directing care of individuals with chromosome 18 abnormalities.

### P03.091

#### Copy number mutations on chromosome 17q24.2-q24.3 linked to congenital generalized hypertrichosis terminalis with or without gingival hyperplasia

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Congenital generalized hypertrichosis terminalis (CGHT) is characterized by excessive universal growth of pigmented terminal hairs. In the present study, we describe three Han Chinese families with CGHT and a sporadic case with extreme CGHT and gingival hyperplasia. We performed linkage analysis in a large four-generation family and identified a CGHT locus at chromosome 17q24.2-q24.3. We then carried out copy number analysis with the Affymatrix Genome-Wide Human SNP Array 6.0 and found nonrecurrent microdeletions in three CGHT families and a larger microduplication in the sporadic case with extreme CGHT and gingival hyperplasia. We validated all the copy number variations (CNVs) by real-time quantitative PCR, and showed that the microdeletions segregated with the disease phenotype in the three families and the microduplication in the sporadic case was *de novo* in origin. These CNVs shared a common overlapping genomic region encompassing the mitogen-activated protein kinase kinase 6 gene, MAP2K6. CNV at the MAP2K6 locus is not reported in the public database and was not detected in normal Han Chinese population. Taken together, our results strongly suggested that the CNVs on 17q24.2-q24.3 were pathogenic copy number mutations responsible for CGHT with or without gingival hyperplasia. This report identifies CGHT as a genomic disorder and might suggest an important functional role of the MAP2K6 gene in hair growth control.

### P03.092

#### De Novo Interstitial deletion 7p15.1p21 Encompassing the TWIST1 Gene in a Boy with Craniostenosis, Marked Lacuna Skull and Facial Dysmorphism but Without Limb and Spine Defects

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A baby boy, delivered at 41 weeks +3/7 days gestation to a healthy 29 year-old G1P0 after an essentially unremarkable pregnancy, was noted to have strikingly abnormal skull shape, abnormal fontanelles and cranial sutures as well as facial dysmorphism that included abnormal anterior hairline, supraorbital creases below and above the eyebrows, shallow orbits, a deep transverse crease over the nose bridge and an upturned nose with under developed nares. The ears were simple with prominent superior and inferior crus of antihelix and the stem of antihelix. The mouth was held open with tented upper lip. The palate was extremely narrow but without clefting. Microretrognathia was evident. He also had an extremely anteriorly placed anus. The genitalia were otherwise normal. The rest of the clinical examination, including the extremities, was normal. Head CT and MRI revealed craniostenosis of the coronal, lambdoid and metopic sutures and partial fusion of the sagittal suture, a fenestrated lacuna skull with ventriculomegaly and a hypoplastic corpus callosum. Skeletal survey showed no spine and limb anomalies. He had an abnormal male karyotype 46,XY,del(7)(p15.1p21) but parental karyotype was normal. The deleted region encompasses TWIST1 gene among several known and presumably many unknown genes. TWIST1 is known to be involved in Saethre-Chotzen syndrome, a condition characterized by craniostenosis, facial asymmetry, abnormal ears and digital anomalies, and less commonly with parietal foramina, radioulnar synostosis, cleft palate and congenital heart malformation. This case exhibited many features of SCS but without spine and limb anomalies, although several novel features were also noted.

### P03.093

#### New case of interstitial 1q44 microdeletion and confirmation of a critical region for corpus callosum abnormalities.

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We identified by array-CGH (oligonucleotides 105K, Agilent Technologies) an interstitial 1q44 deletion, spanning about 2.1 Mb, in a patient with mental retardation, dysmorphic features and brain abnormalities. This deletion arose *de novo*. The proposita was the only child of healthy non-consanguineous parents. She was born at term: birth-weight was 3060 g (M), length 49 cm (M), and OFC 35 cm (M). Family history is non-contributory. At 30 months, height was 82 cm (-2.5 SD), weight was 11 kg (-1 SD) and OFC was 47 cm (-1 SD). Clinical features included brachycephaly, facial dysmorphism with deep set eyes, synophrys, horizontal eyebrows, prognathism, clinodactyly of fifth fingers, flat feet. She walked unaided at 24 months and had no speech. She had clonic seizures. Brain MRI showed hypoplasia of the anterior part of corpus callosum, and venous angioma in the right frontal lobe. Electroencephalogram showed central epileptic spikes predominating on the left side. This patient shows common clinical features to the individuals with 1q44 deletions. This deletion involves 10 genes and comprises the critical region for corpus callosum abnormalities described by van Bon BWM et al., J Med Genet 2008, 45:346-354.

### P03.094

#### A case of partial trisomy/monosomy of chromosome 8p associated with autism and epilepsy defined by genome array-CGH

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Autism is a neurodevelopmental disorder with early childhood onset and a prevalence of as much as 5/10,000. Symptoms that may contribute throughout life include qualitative impairments in reciprocal com-

munication and social interaction, as well as repetitive and stereotyped behavior.

We report on a case of a 8-year-old male with partial trisomy 8p(22;23.1)/partial monosomy 8p( 23.2;pter) associated with autism, mild dysmorphic features, epilepsy and moderate learning disability. The cryptic deletion has been detected by Genome array-CGH.

Although mental retardation is a common finding in patients with mosaic trisomy 8 or partial trisomy of various regions of chromosome 8, only two cases associated with autism have been reported so far and. In our case a cryptic deletion is also present. To the best of our knowledge the present case represents the first description of simultaneous presence of dup/del 8p. Clinical manifestations were mild compared to other patients with duplication of the same region of chromosome 8. Although there has been no strong evidence for linkage on chromosome 8 in any of the genome-wide linkage studies so far, the possibility that this segment includes genes involved in the etiology of autism should be further explored.

#### P03.095

#### Characterization of a supernumerary marker chromosome using classical cytogenetics and aCGH method : example of a partial 9p,15q trisomy and genetic/phenotypic correlations

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We report on a 1 year old patient with mild dysmorphism and developmental delay.

Born at term with normal growth parameters, he demonstrated since the age of 6 months, growth and weight retardation. Delay in motor milestones was observed as the child did not sit alone at 9 months. He had also reduced social interactions.

G-banding showed the presence of a supernumerary chromosome (47,XY,+mar), composed, as revealed by high-resolution aCGH, of 27 Mb from region 9p24.3 to 9p21.2 and 12,3 Mb from 15q11.2 to 15q13.3. This derivative chromosome [der(15)t(9;15)(p21.2;q13.3)] was subsequently found to derive from a maternal reciprocal translocation, very likely originating from a meiotic missegregation of 3:1 tertiary type.

Partial trisomy 9p21.2-9pter is not well defined but two patients with an intrachromosomal duplication of chromosome arm 9p [dup(9)(p21p24)] demonstrated classical clinical manifestations of 9p trisomy with, in one case, features overlapping with Coffin-Siris syndrome.

Duplication of the 15q Prader-Willi /Angelman syndrome region is mainly associated with autistic behavior, mental retardation and developmental delay. Most reported cases with associated clinical symptoms have been linked to extra copies of 15q11-q13 from maternal origin, paternally-derived duplications being generally associated with a normal phenotype. Our results also support this observation.

Presence of potential extra copies of numerous genes from chromosome 9p in addition to the duplicated 15q region renders a clinical prognosis difficult. Further assessments and clinical evolution of the patient will help to better define the spectrum of effects of the diverse duplicated genes on the phenotype and on their interactions.

#### P03.096

#### Array CGH analysis pinpoints to autosomal recessive syndromes due to genes outside the rearranged region

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A cohort of 213 mentally retarded patients has been analyzed by array-CGH with a resolution of about 100Kb (Agilent 44K). Thirty six cases (17%) were considered positive using the following criteria: i) de novo non polymorphic rearrangements (13/213 or 6%)(Am J Med Genet A.2008;146A:1994-8 and A.2007;143A:858-65; Eur J Med Genet 2007;50:21-32 and 2007;50:315-21 and 2008;51:409-16); ii) either inherited (15q11.2q13.2) or de novo rearrangements of known syndromes (19/213 or 9%); iii) either inherited or de novo rearrangements in susceptibility regions (16p11.2, 15q13.3) (4/213 or 2%). Since it has been proved that in mammals rearrangements may alter expression of genes lying up to 10Mb from the breakpoints, we re-analyzed our cohort paying attention to the surrounding regions. We describe here three cases (1.4%) with a possible autosomal recessive syndrome due to genes outside the rearranged region. In a sex reversal 46XX

male a 1.8Mb inherited duplication in 17q12 lays 4Mb upstream of the 17beta-hydroxysteroid dehydrogenase 1 gene that encodes 17HSD1, which catalyzes the final step of testosterone biosynthesis. In a sex reversal 46XY female a 0.2Mb inherited deletion in 17q12 lays 7Mb apart upstream of the above mentioned gene. In a Cohen-like patient (microcephaly, truncal obesity tapered fingers with brachidactyly, short stature, evocative facial gestalt) a 2.1Mb de novo deletion in 8q22 lays 1Mb downstream to the Cohen gene. In order to prove the hypothesis of recessive syndromes due genes outside the rearranged region mRNA analysis and mutation analysis on the candidate genes is ongoing.

#### P03.097

#### Screening of 314 patients with mental retardation and /or multiple congenital abnormalities by array-CGH: experience of Geneva-Lausanne centers.

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Array-Comparative Genomic Hybridization (aCGH) allows a high resolution whole genome analysis of copy number changes and can reveal submicroscopic deletions and duplications.

We present the results of aCGH analysis compiled from two Swiss centers including 314 probands with mental retardation, developmental delay, and/or congenital malformations. An oligo aCGH with an average coverage of 40 kb (Agilent 244K) was used. *De novo* Copy Number Variants (CNV) were identified in 12.3% of the cases including 28 deletions, 11 duplications, and 2 terminal deletions associated with proximal duplications. The copy number variants ranged from 39 kb to 13.7 Mb. In 126 (46%) out of the 314 examined patients, CNV were detected and required further analysis of parental genomic DNA. In 42 of these cases, the observed variant (range 30 kb to 7.3 Mb) was found to be inherited from a healthy parent.

Our cohort also includes six cases of *de novo* apparently balanced translocations in which aCGH did not detect cryptic genomic imbalances at the breakpoints. As positive controls, 19 cases of unbalanced karyotypes analysed revealed large deletions and duplications. We conclude that aCGH substantially contributes to the diagnostic evaluations of patients, but interpretation of the results requires the collective efforts of many laboratories and the examination of large numbers of cases and controls.

#### P03.098

#### Monozygotic twins discordant for submicroscopic chromosomal anomalies detected by array CGH

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Although discordant phenotypes in monozygotic twins used to be considered as an exception, an increasing number of reports indicate that this phenomenon is not so rare. Most of reported patients have numerical chromosomal anomalies, with only few cases having structural chromosomal anomalies. Here, we report on the clinical and cytogenetic details of 4-year-old female monozygotic twins with discordant phenotypes.

Twin 1 exhibited global developmental delay with walk at 35 months, absence of speech, and hyperactivity. Twin 2 had an autistic spectrum disorder without motor delay. Extensive investigations including blood karyotype, metabolic screening and brain MRI were normal in both twins.

Molecular karyotyping in twin 1 identified a 2p25.3 deletion, further confirmed by FISH analysis on leukocytes in all cells. Interestingly, array-CGH was normal in twin 2 but FISH analysis performed on leukocytes using the same probes showed mosaicism with 33 % of deleted cells, 33 % of duplicated cells, and 33% of normal cells. Genotyping confirmed the monozygosity of the twins and ruled out uniparental di-

somy for chromosome 2. We propose that the discordant chromosome imbalance may be due to a mitotic non-allelic recombination occurring during blastomeric divisions of a normal zygote. Such event will result in 3 distinct cell populations whose proportion in each embryo formed after separation from the inner cell mass, may differ, leading to discordant chromosomal anomalies between twins. To our knowledge, this is the first report of monozygotic twins with discordant phenotypes ascribed to a distinct submicroscopic rearrangement detected by array-CGH.

#### P03.099

##### Detection of low-level mosaisms by array CGH

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Mosaic chromosomal aneuploidy has long been recognized as a cause of abnormal prenatal and postnatal development. Molecular karyotyping or genome wide array comparative genomic hybridization (CGH) is a technique enabling a rapid screen of the genome at high resolution. Yet, the detection limit of array CGH to detect and evaluate low-level mosaic gains or losses is not known. Here we report a significant improvement of the array CGH limit of detection, with the analysis of "artificial" chromosome mosaisms, created by making mixtures in varying proportions of genomic DNA samples of a normal diploid adult female and an adult male with trisomy 21. For each mosaic sample, array CGH was performed using commercial BAC arrays (CytoChip), and housemade 1Mb arrays. We describe a statistical power analysis methodology to assess the detection limit of the technology. Our data demonstrate that it is possible to detect whole chromosome deletions to as low as 2%. The results of the array CGH analysis were evaluated using Students t-tests and frequency distribution plots. Thus, array CGH, which is based on genomic DNA extracted directly from uncultured peripheral blood, is an excellent method to detect low-level mosaic chromosome abnormalities.

#### P03.100

##### Recurrent microdeletion at 17q12 as a cause of Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome: two case reports

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Mayer-Rokitansky-Kuster-Hauser syndrome (MRKH; OMIM 277000) is a rare disorder, with a prevalence of 1:4,500 female births, characterised by congenital aplasia of the uterus and upper part of vagina, due to anomalous development of Müllerian ducts. MRKH can be isolated or associated to other malformations, including renal, skeletal, hearing and heart defects. We report on two patients affected by MRKH in which array-CGH analysis disclosed the deletion of the same 1.5 Mb segment at 17q12 region. The first had complete absence of uterus and vagina, while the second presented with agenesis of the upper part of vagina, right unicornuate uterus and non cavitating rudimentary left horn associated with bilaterally multicystic kidneys. This deletion covers the candidate gene *TCF2* and represents a recurrent rearrangement mediated by segmental duplications, already reported in individuals with developmental kidney abnormalities and diabetes. *TCF2* is the causative gene of Maturity-Onset Diabetes of the Young type 5 (MODY5, OMIM137920) a disorder encompassing a wide clinical spectrum which includes, in addition to MODY, abnormal renal development. Interestingly, congenital malformations of the genital tract, such as bicornuate uterus and Müllerian aplasia in females and epididymal cysts and bilateral agenesis of vas deferens in males, were reported in patients with point mutations and/or deletions of *TCF2*. We carried out *TCF2* gene screening in a group of 20 non-deleted

MRKH subjects by direct sequencing and no pathogenic mutations were found. The present results suggest that 17q12 is a candidate region for a subset of MRKH syndrome individuals, with or without renal defects.

#### P03.101

##### Comparison of 1 Mb BAC array and 105K oligo array in a clinical diagnostic setting.

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Over the last five years, 1 Mb resolution BAC arrays have been implemented for the clinical diagnosis of patients with mental retardation and/or congenital malformations. Several higher resolution arrays have since been developed. Here, we evaluate the clinical value of a higher resolution oligonucleotide based array platform. The array contains 105,000 oligonucleotides (105K). The array consists of a backbone coverage of one oligo every 30kb and, in addition, targeted over 200 syndromic regions and over 400 clinically relevant genes. 98 patients referred to the CME laboratory for aCGH were analysed using the Syndrome Plus array, and the results compared to those obtained from the in-house 1Mb BAC array currently in diagnostic use. 31 aberrations were detected in 23 patients using the 1Mb BAC array, providing a diagnostic yield of ~23%. All aberrations were confirmed on the 105K array. Currently, we are determining the sensitivity and specificity of this array to eventually determine whether the arrays provide a higher diagnostic yield. The results of this analysis will be presented.

#### P03.102

##### Deletion of 7.9 Mb in the region 15q21.3q22.31 identified with SNPs array

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Unlike the small proximal 15q deletions causing Prader-Willi and/or Angelman syndrome, distal or interstitial of long arm of chromosome 15 have rarely been described. Here we describe a 8-year-old male with a several mental retardation, speech delay and hypotonia. At birth he was hospitalized in neonatology for abdomen significantly increased in volume as a result of polycystic kidney. For this reason was performed a right nephrectomy. At 5 1/2 months to begin seizures, there was a slowdown in the stages of development and 18 months starting antiepileptic therapy. Physical examination revealed dysmorphic features (plagiocephaly, hypertelorism, telecanthus, palpebral fissures slanting down, small nose with wide nasal bridge and bulbous tip, anteverted nares, short philtrum, anteverted lips, malformed teeth, large and low-set ears). An electroencephalogram revealed a poorly organized pattern by age. MRI revealed a smaller corpus callosum and a slight increase in the cerebral ventricles, but no signs of infection or stroke were present. ENT examination revealed a laryngomalacia. An ultrasound examination revealed the suspected diagnosis of intestinal dysplasia. The chromosomal analysis was performed on blood and revealed a 46, XY karyotype. SNP array analysis, from a whole blood sample, was carried out using SNPs array using the HumanCNV370-Duo platform (Illumina, San Diego, California) according to manufacturer's protocol, and identified in our patient a 7.9 Mb deletion of chromosome 15q21.3-p22.31. Up to now three other cases with a similar 15q monosomy have been reported, but the present case presents a much smaller deletion.

#### P03.103

##### Clinical and molecular characterization of two patients with a 6.75 Mb overlapping deletion in 8p12p21 with two candidate loci for congenital heart defects.

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Several 8p syndromes associated with deletions in different regions on 8p have been reported, although most cases are terminal deletions encompassing the 8p23 region. It has been proven difficult to

link the separate clinical features to specific chromosomal locations since the various different regions were just partly overlapping and not highly specific. However, critical regions for some features have been delineated, such as heart defects assigned to 8p23.1, encompassing the *GATA4* gene and *GNRH1* in 8p21.2 as a candidate gene for hypogonadism. To further delineate the phenotypic spectrum of interstitial deletions proximal to 8p23, we studied the clinical and molecular characteristics of two patients with a 6.75 Mb overlapping interstitial deletion in the 8p12p21 region and compared these with 17 previously published cases with an overlapping deletion. The most common characteristics of interstitial deletions of proximal 8p are developmental delay, postnatal microcephaly and growth retardation. Other frequently reported findings are hypogonadism associated with haploinsufficiency of *GNRH1* and ocular problems. Congenital heart anomalies are also common and might be due to haploinsufficiency of *NKX2-6* and/or *NRG1*, given that *GATA4* is not involved in these proximal deletions. The aforementioned clinical characteristics should be considered in the care of patients with a proximal interstitial 8p12p21 deletion.

#### P03.104

##### A patient with de novo duplication of 13(q31.1-->qter)

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We present a 6 month-old boy, who is the third child of consanguineous Turkish parents. He was born by spontaneous vaginal birth at 40 weeks, and referred to our clinic due to facial dysmorphic features and abnormalities.

On his physical examination; weight was 8500 g (<3th centile), height was 80 cm (10th centile) and head circumference was 43.5 cm (<3th centile). He had typical features including upswEEP, brachycephaly, prominent glabella, long eyelashes, dysplastic ears, high arched palate, retrognathia and unilateral cryptorchidism. His ophthalmologic examination showed bilateral leukocoria, iris colobomata, retrotentorial membrane and retinal detachment. Echocardiographic findings indicated perimembranous VSD. His karyotype revealed 46,XY,der(13) by GTG banding. The chromosome abnormality occurred *de novo*, as both parents had normal karyotypes. To determine the origin of the extra segment, we performed microarray analysis with a 50 K GeneChip array and it manifested a duplication in the q31.1-qter of chromosome 13. Accordingly, the karyotype was described as 46,XY,dup(13)(q31.1-->qter)

In this presentation, the clinical phenotype regarding the literature will be discussed, and we expect the presence of the pure dup (13)(q31.1-->qter) in our patient could provide an opportunity to delineate the phenotypic features due this partial trisomy.

#### P03.105

##### Partial trisomy 19p in a girl with general retardation and dysmorphic signs

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Introduction: Array CGH is an essential tool for the detection of microscopically invisible chromosomal aberrations in retarded and dysmorphic children. In a female child with general retardation and facial dysmorphisms a microduplication 19p13.3 was identified. Chromosome analysis in the parents showed that the mother is carrier of a small balanced translocation t(14;19)(p11.1;p13.3).

Case report: AF, the second child of healthy parents, was born in May 2005 in the 38<sup>th</sup> week of gestation (38+0). She was small for gestational age (birth weight 1.825g, length 43cm, head circumference 28cm - all < 3<sup>rd</sup> percentile) and showed facial dysmorphisms. At the age of one year chromosome analysis revealed a normal female karyotype (400 band stage). AF was presented again at the age of 3 years and 2 months at our genetic counselling unit. Weight was 10,8 kg, height 93cm and head circumference 38cm. She showed a developmental delay and was able to speak only few words.

Methods and results: Oligonucleotid-based array CGH on Agilent 44K arrays revealed a 4,68Mb microduplication in 19p13.3 deriving from a cytogenetically balanced translocation t(14;19)(p11.1;p13.3) in the

mother.

Conclusions: Partial duplication of 19p13.3 is a rare condition with only few case reports published. We overview the known published cases and give a comparison with our patient. The influence of the known genes in the duplicated region will be discussed

#### P03.106

##### Identification of a Deletion on Chromosome 3p(12.3) by Whole Genome Analysis in a Discordant Monozygotic Twins with a Tail and Multiple Congenital Anomalies

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Human tail (caudal appendage) is a rare dysmorphic feature and etiologic mechanisms of human tale are not well understood. Here we report monozygotic twin brothers who are discordant for the caudal appendage and multiple congenital anomalies. The index case was referred to the hospital prematurity and intrauterine growth retardation. He was born to born to a healthy nonconsanguineous parents at 27 weeks old. On his physical assessment on admission there were micrognathia, beaked nose, hypospadias and caudal appendage. Caudal appendage was 2,5-cm-tail-like structure which was surrounded by a soft tissue mass at the level of S4. Pathologic examination of the excised specimen revealed 1.2x1.1x1.0 cm, mature adipose tissue, connective tissue and hyaline cartilage tissue bone tissue. Echocardiography revealed juxtaductal aorta coarctation. Karyotype analysis showed 46,XY. FISH analysis for 22q deletion was negative. Monozygosity showed by 16 microsatellite markers. We performed genome wide copy number analysis to monozygotic twins. We hybridized each other for arrayCGH. By 384K whole genome analysis two neighboring probes at 3p12.3 has got high Log2 ratios for deletion. High resolution of chromosome 3 array confirmed ~ 710 Kb deletion where ZNF717, FRG2C and FAM86D genes are mapped. Although it has been reported to be a variable region and epigenetic mechanisms are also blamed in the etiology of human tail, this case is the first report presenting a CNV in this region with multiple anomalies.

#### P03.107

##### Characterization of a double ring chromosome 4 mosaicism associated with bilateral hip dislocation, cortical dysgenesis, and epilepsy

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We present the clinical and cytogenetic findings in a Turkish child with a *de novo* mosaic double ring chromosome 4, (46,XY,r(4)[83]/45,XY,-4[6]/47,XY,r(4),+r(4)[5]/48,XY,r(4),+r(4),+dic r(4)[1]/46,XY[5]) karyotype. The propositus is a 20-month-old male who was the product of the first unremarkable pregnancy of nonconsanguineous parents of 19-year-old mother and 28-year-old father. The baby was delivered vaginally at term and, at birth, weight was 1,700 g (<3rd centile), length was 46 cm. The baby had feeding difficulties and vomiting problems. He started walking at 2 years of age and language delay was seen. While facial appearance was normal, ears were large, dysplastic, and had bilateral less configuration in the helices. The hands showed bilateral clinodactyly of the 5th fingers. The patient's lower extremities had bilateral hip dislocation. He was mildly mentally retarded with epilepsy starting at 8 months of age. Magnetic resonance imaging of the brain showed cortical dysplasia while EEG test was normal. Renal sonography and ophthalmic examination were normal, too. The karyotype was refined to 46,XY,r(4)(::p16.3->qter::)[67]/46,XY,r(4;4)(::p16.3->qter::p16.3->qter::)[2]/46,XY[3] by multicolor banding (MCB) technique. The Wolf-Hirschhorn critical region was preserved. By array CGH the size of the deletion was delineated as 900 kb in 4p16.3. Additionally, two small unreported copy number variants were detected on other chromosomes which do not contain genes associated with developmental delay. To the best of our knowledge the reported case is unique and provides further insights in complex rearrangements present especially in the first trimester.

cially in chromosome 4. Supported in parts by Prochance 2008 of the Friedrich Schiller University Jena 21007091 and DFG (LI 820/14-1).

#### P03.108

#### Girl with left hemiatrophy reveals confined mosaicism for r(13) in fibroblasts

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In the background of pigmentary skin anomalies or asymmetry (usually encountered in the form of hemihypertrophy) when combined with seizures and mental retardation, mosaicism for somatic chromosomal rearrangements should be searched for.

We report a seven-year-old girl with total left hemiatrophy, microcephaly, facial dysmorphism and neuromotor retardation. The face was asymmetric with ipsilateral microphthalmia and iris coloboma. Hypertelorism and low-set ears were also noted. Karyotype was normal in 150 metaphases from peripheral blood lymphocytes. Cytogenetic analysis of the cultured skin fibroblasts revealed mosaicism for ring chromosome 13, 46,XX/46,XX,r(13)(p11q14) [35/15] on the left side and [12/8] on the right side of the body. By FISH studies using D13S1825 probe specific to the q telomere of the chromosome 13, no signal on the ring chromosome was obtained. The uncultured peripheral blood cells were checked out with this probe, and in each of the 300 interphase nuclei, 2 signals were observed.

Hemiatrophy is a rare clinical finding, and not amongst the clinical findings associated with partial deletions of 13q. To the best of our knowledge, our patient is the second case for somatic mosaicism of 13q, but unique for hemiatrophy.

#### P03.109

#### Chromosomal aberrations among patients with mental retardation

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Cytogenetic investigation among children with mental retardation of different degree was performed. In total of 40 children (fourteen girls and twenty-six boys) aged 4-14 years were examined. For the most part (80%) mental retardation coupled with congenital malformations and dysmorphisms.

Cytogenetic analysis of G-differential staining chromosomes (G-bands) has revealed abnormalities among 30 % patients. The numerical abnormalities of sex chromosomes were detected among three patients (25%). It was represented by monosomy and polysomy of X-chromosome. Two patients (17%) had Fragile X mental retardation syndrome (OMIM 300624). Structural balanced (25%) and unbalanced (33%) chromosomal aberrations were diagnosed in other patients (58%). In our study we found rare chromosomal rearrangements: 46,X,t(X;13)(q11;q12), 46,XX,del(18)(p1.1), 46,XX, dup(8)(q12q13). Thus, our investigation suggested that patients with mental and speech disabilities, autism and epileptic syndrome needs in additional examinations, which permit hereditary to diagnose hereditary pathology.

#### P03.110

#### 5p duplication syndrome: a rare multiple congenital anomaly-retardation syndrome caused by partial duplication (5) (p15.2-p12) combined with partial deletion (5) (pter-p15.31)

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We report on an infant with multiple congenital anomalies, developmental delay, abnormal neurological and dysmorphic signs.

He is the first and only child of a healthy nonconsanguineous parents with no positive family history regarding congenital diseases. His phenotype is characterised by failure to thrive, developmental retardation, severe muscular hypotonia, congenital heart anomaly, agenesis of corpus calosum, pronounced macrodolichocephaly, unusual face with hypertelorism and bulbous nose with flat bridge, full lips, long fingers, limb abnormalities and hearing loss. With combination of banding studies and FISH analyses the karyotype 46, XY, der (5)dup(5) (p15.2-p12) del(5) (pter-p15.31) was identified. This so far unpub-

lished partial duplication 5p combined with partial deletion 5p of de novo origin is the cause of described clinical picture that is typical for 5p duplication syndrome since the critical 5p13 region is also included in the duplication.

#### P03.111

#### Report of a dysmorphic case from IRAN with a new finding, and structural abnormality in the long arm of chromosome 1

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Background: Partial trisomy of 1q42 is one of the structural Chromosome abnormalities with a distinctive phenotype.

Material and Method: Here in we report a 1-year-old Iranian girl referred to our genetics center because of neuro-developmental delay and dysmorphic findings.

Cardinal features were: trigonocephaly, microcephaly, spasticity, sunken eyes, prominent forehead, low set and malformed ears (with posterior rotation and abnormal helix), micrognathia, long philtrum, carplike mouth, frontal bossing, high palate, high nasal bridge, high arched eyebrows, short neck, strabismus, and asymmetric face and locked jaw, abnormal and small hands and feet, brachydactyly of fingers and toes, flat feet, congenital heart disease, dysplastic nails, simian crease in right hand and abnormal sole in the left hand.

Chromosme study: according to the MR, and MCA we carried out chromosome analysis by high resolution GTG banding technique. The result was a structural abnormality, a duplication in 1q42 region. Parents were investigated and they were normal.

Conclusion: our study showed that this chromosome Abnormality was de novo in this case. So, we should consider structural and numerical chromosome abnormalities in the patients, with MCA+MR. Microcephaly is a new finding for this locus, and was not reported before.

#### P03.112

#### A Case with Mosaic Ring Chromosome 18

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The 11 year old case was the second pregnancy and the second child of the family, was born full-term with a birth weight of 3100 g. At the time of the birth, the age of both parents were 24. Growth retardation of the case was realized at the age of 1,5. The case with congenital malformation, mental retardation, short stature, high palate, pectus excavatus, big and low set ears, bilateral strabismus, distinct front incisors, hypertelorism, flat broad nose root, wide nostrils, long frenulum, pes planus in both feet, overlapping of the second toe onto the third toe, frequent infection, speech defect was operated at the age of two due to PDA. The karyotype of the case was detected to be 46,XX/46,XX,r(18) (25% mosaic) in the chromosome analysis performed. The classical type of the ring chromosome formation is by the fusion of breaks occurred in both arms of the chromosome and loss of the distal fragments. The ring of the chromosome 18 is rare among ring chromosomes. The typical clinical signs of the 18p and 18q Syndrome rate depend on the size of the deletions in 18p and 18q. r(18) phenotype is characterized by growth retardation, mental retardation and non-specific abnormalities. Facial dysmorphism and malformations may also be associated. As a result of FISH analysis performed using Cep 18 (Aqua) ve Telomer 18q (Red) probes, the karyotype of the case was verified to be 46,XX/46,XX,r(18) (25% mosaic).

#### P03.113

#### Mandibular dysmorphology in prenatal trisomy 18

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Introduction: Among 2nd trimester aborted foetuses, trisomy 18 is one of the most common autosomal trisomies. By ultrasonography it may be difficult to distinguish between trisomy 18 and trisomy 13. According to Keeling (1994), the prenatal trisomy 18 head is globular in shape, with hypertelorism, a broad up-turned nose, micrognathia and backwards sloping abnormal ears. Short nasal bones have also been reported.

The purpose of this preliminary study is to add phenotypic character-

istics to prenatal trisomy 18 by evaluating the mandibular morphology, easily recognisable by ultrasound.

**Material and methods:** Lateral faxitron radiographs of 13 postmortem trisomy 18 foetuses (mean GA=17 weeks) were compared with similar radiographic exposures of 8 postmortem normal foetuses (mean GA=16 weeks). The mandibular morphology in the lateral view was expressed by measuring the mandibular angle (also named the gonial angle), which is the angle between the tangent to the mandibular basis and the tangent to the posterior contour of the mandible.

**Results:** Gonial angle in trisomy 18: Mean 154.4° (SD=5.8). Gonial angle in normal foetuses: Mean 135.0° (SD=6.1). Tested by unpaired t-test P<0.001.

**Conclusion:** The size of the gonial angle, measured in this preliminary study, is significantly larger in trisomy 18 when compared to normal. The gonial angle is easy to recognize and measure during ultrasonography and accordingly, this enlarged angle seems to be a new valuable dysmorphic feature in trisomy 18.

**References:** Keeling JW *Fetal Pathology*. Churchill Livingstone, 1994.

#### P03.114

##### Report of Two Iranian Patients with 13q partial monosomy, mental retardation and dysmorphism

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We describe two patients referred to our center because of mental retardation (MR) and dysmorphism.

The first Patient is a five year old boy with non related parents. He has moderate to severe MR and developmental delay. His clinical features include IUGR, microcephaly, hypospadias, club foot, speech delay, ptosis, syndactyly, equine valgus, strabismus, cleft palate, simple ears, micrognathia, and high nasal bridge. Routine cytogenetics investigation showed a terminal deletion of the long arm on chromosome 13, described as 46, XY,del(13)(q32)de novo.

The second patient is a twelve year old girl with consanguineous parents. She has moderate to severe MR. Her clinical features include developmental delay, upper limb muscle weakness, high nasal bridge, wide mandibular angle, high arched palate, thin and long face, down slanted palpebral feature, low hair line, webbed neck, expressionless face, and scapula alata. Routine karyotype analysis showed an interstitial deletion of the long arm of chromosome 13, described as 46,XX, del(13)(q12.3q14.3)de novo. Most of the clinical features of these two patients overlap with other reported cases of 13q partial monosomy. Complementary works for further elaboration of the deleted segments are underway.

#### P03.115

##### A report of a new case with de novo 18 q deletion syndrome

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A deletion involving the long arm of chromosome 18 may be associated with a variable spectrum of phenotypic findings. Size of deletion appears to correlate with severity of phenotype. The major clinical features are postnatal growth retardation and short stature, mental retardation, typical facial dysmorphia (midfacial hypoplasia with deep set eyes, ocular anomalies, epicanthal folds, carp-shaped mouth, cleft lip/palate, proeminent antihelix and/or tragus, other ears anomalies), behavioural problems, hypotonia and poor coordination. Other anomalies are: short extremities, hygonadism, urethral reflux and seizures.

We present a case with the novo 18q deletion syndrome: S.C., male, 16 years old who present an episode of epistaxis and hematemesis. We note normal pregnancy. He was diagnosed with congenital heart defect (VSD, pulmonary hypertension), afebrile seizures and Ig A deficiency at 7 months old. Clinical evaluation at 16 years old revealed:

obesity, craniofacial typical dysmorphia (microcephaly, flat midface, down-slanted palpebral fissures, proeminent nose, prognathism, thin upper lip, dental anomalies), short neck, brachydactyly of hand and feet, hypogonadism (micropenis and cryptorchidism), hypopigmentation (vitiligo) and severe mental retardation. Paclinical evaluation: ECG revealed prolonged QT interval; EEG indicate abnormal brainwave. Ecocardiography: subaortic SDV (Septal Ventricular Defect), tricuspid insufficiency, pulmonary arterial hypertension, cardiomyopathy. The karyotype was abnormal: 46,XY,del(18)(q21.3→qter). The karyotypes of both parents are normal.

In conclusion, the patient presents a de novo anomaly (18 q terminal deletion). The risk of the parents to have a new affected child with the same anomaly is very low.

#### P03.116

##### 18q deletion syndrome - case report

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18q- syndrome represents a rare deletion syndrome characterized by mental retardation and congenital malformations. In a majority of patients cytogenetic studies reveal terminal 18q deletions of variable size, with only few patients having interstitial deletions or more complex cryptic rearrangements.

We report on a 3 year-old boy with mental retardation, cerebral dysmyelination and dysmorphic features, bearing a distal deletion of 18q: 18q21-qter.

Karyotype analysis was performed on peripheral blood lymphocytes, by GTG banding. Painting probe for chromosome 18 and bacterial artificial chromosome probe (BAC-FISH probe) for 18q23 (RP11-248M19) were applied for molecular characterization.

Clinical features at presentation were severe mental retardation, severe speech delay, hypotonia, facial dysmorphia (broad forehead, broad nasal bridge, thin lips, micrognathia) and clynodactyly of 5<sup>th</sup> finger. Brain MRI showed cerebral myelination defects. Severe hypogammaglobulinemia, responsible for repeated febrile episodes, was also detected.

Chromosomal studies revealed a deletion of the distal fragment of chromosome 18q ranging from band q21 to qter. Painting FISH for chromosome 18 ruled out cryptic rearrangements involving other chromosomes. The terminal 18q deletion was confirmed by FISH with subtelomeric probe RP11-248M19.

In conclusion, a large terminal deletion of 18q was detected in a patient displaying major phenotypic features of 18q- syndrome. Further delineating the deleted region in our case might contribute to a better phenotype-genotype correlation.

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#### P03.117

##### 5q12 proximal deletion : delineation of a phenotype including ocular findings.

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Array-CGH enables the detection of submicroscopic chromosomal deletions and duplications and lead to an accurate delineation of the imbalances, raising the possibility to do genotype-phenotype correlations, to identify minimal critical regions and candidate genes for a pattern of clinical features. We report here three patients sharing common clinical features (psychomotor retardation, coarse facies and ocular motricity anomalies), with a proximal 5q deletion identified by oligo array-CGH. The deletions are from 5.7 to 15.3-Mb in size and occurred de novo. A common 2.63-Mb deleted region can be defined in 5q12

(59.390.122 to 62.021.754 Mb for 5pter, hg18). Proximal 5q cytogenetically visible deletions including 5q12 have been reported in patients with psychomotor retardation and multiple congenital anomalies. No recognizable phenotype has been described but eye findings (ptosis, epicanthus, astigmatism, esotropia and nystagmus) were observed in these interstitial deletions. The common deleted region in our cases includes 11 genes, some of them are listed in the OMIM database. *KIF2A*, which encodes a kinesin superfamily protein, is particularly interesting as it plays an important role in the suppression of the growth of axonal collateral branches and is involved in normal brain development. Ocular findings seem to be common clinical features, even if they are not specific, in the 5q12 microdeletion. Identification of additional cases of deletions involving the 5q12 region will probably allow more accurate genotype-phenotype correlations.

#### P03.118

#### Complex eye malformation in a fetus with trisomy 6p22p24

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We report a fetus with additional material on chromosome 6p detected on routine amniocentesis for maternal age in a 39 years old woman. Fetal ultrasonographic examination was normal. Both parents had normal karyotypes. A medical termination of pregnancy was performed at 31 WG. At autopsy, the fetus was eutrophic and had no major visceral anomaly. The pancreas appeared short and the right lung was bilobed. In the brain, olfactory bulbs were absent and there was a fusion of meningeal membrane in the anterior part of cortex. At microscopic examination, we found a duplication of the ependymal canal in the lumbar region of the spine and bilateral eye coloboma with dysplastic retina. The karyotype was: arr cgh(RP11-304M10---RP1-67M12)x3 using array-cgh BAC (4400 clones - Perkin-Elmer Cytochip). The 6p22p24 region contained several genes, in particular *KIF13A* and *NUP153* (nucleoporin) genes, whose gain is known to be involved in eye malformations. We hypothesize that overexpression of these genes may play a role in the ocular anomaly observed in our case.

#### P03.119

#### A new case of chromosomal translocation: t(4;10)(q35;q22.1)transmitted from mother to daughter

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We presented a new case with chromosomal translocation, discovered first in a plurimaleformate child. The girl is the first child of a healthy, young, nonconsanguineous couple. The delivery was produced at term, but child presented an intrauterine growth retardation (1400 gr weight, 46 cm height and 28 cm cranium perimeter) neonatal jaundice and respiratory distress. The APGAR score was 4. The clinical examination revealed: microcephaly, flat face, bilateral microophthalmia (confirmed by ophthalmologic examination) short nose, flat, short philtrum, microretrognathia, short neck, bilateral syndactyly of II and III toes, genital hypoplasia, and muscular hypotonia. Cardiologic examination revealed a systolic murmur. Thorax radiography revealed a "wooden shoe heart" with an important left ventricular hypertrophy. Echocardiography indicated a small ventricular septal defect, open *foramen ovalis*, tricuspidian insufficiency and persistence of arterial duct. The karyotype of girl revealed an abnormal chromosomal formula: 46,XX,der(4;10)(q35;q22.1). For establish if the abnormality is *de novo* or inherited we made the chromosomal analysis in parents. The karyotype of father was normal, but mother presented a balanced translocation: 46,XX,t(4;10)(q35;q22.1). Because the moved fragment of chromosome 4 is very small and moved fragment of chromosome 10 represent about half of long arm, we estimate that couple have a 10% risk to have another abnormal child with an important partial 10 trisomy associated with insignificant partial 4 monosomy. For this reason we indicate a prenatal chromosomal analysis in next pregnancies of couple. This case reveals the importance of chromosomal analysis in neonates with plurimaleformative syndrome and parents of children with abnormal karyotype.

#### P03.120

#### Results of 3248 cytogenetic analyses: a retrospective study in karyotype abnormalities

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Center for genetics from Medical faculty in Sarajevo have finished 3248 karyotype analyses from peripheral blood lymphocytes - and using GTG -band technique during last ten years. 360 chromosomopathies were reported, 254 somatic and 53 gonad aberrations. Sy. Turner was the most frequent gonad aberration and was reported in 31 patient, Klinefelter Sy was the second most frequent gonad aberrations and was reported in 12 patients, 4 patients were with 47,XXX karyotype, 3 patients with testicular feminization and 3 patients with fragile X syndrome.

From 254 somatic aberrations, trisomies including mosaic types were reported in 228 cases and 26 were with somatic structure aberrations. The most frequent was Sy Down (Trisomy 21) in total of 216 patients, Sy Edwards (Trisomy 18) in 4 patients, Trisomy 13 in 2 patients and Trisomy 22 in 2 patients. Translocations and deletions were occurring individually and *de novo*, 26 of these patients were identified.

#### P03.121

#### Characterization of a complex chromosomal rearrangement involving chromosome 10.

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Complex chromosomal rearrangements are rare. Here we report on a young baby girl born at 37 weeks of gestation. On clinical examination, the following dysmorphic features were observed: hypertelorism, blepharophimosis, bilateral epicanthus, retrognathia and a short neck. The mouth is small with a long and flat philtrum. She has small and square low-set ears. The fingers are long and thin with a II-IV membranous syndactyly and a II and V clinodactyly. Rocker-bottom feet and a II-III syndactyly were observed. Congenital heart defects (atrial septal defect and ventricular septal defect), abnormal genitalia (clitoris hypertrophy) and bilateral renal dysplasia were diagnosed.

Cytogenetic analyses using G and R banding techniques identified a der(10) chromosome. FISH study using a chromosome painting (wcp 10) probe excluded other chromosomal material in the rearrangement. Further FISH studies using subtelomeric probes showed that on the der(10) chromosome, 10qtel was replaced by 10ptel. In addition an inverted duplication of the 10q25.3q26.2 region was observed. Further studies revealed that the der(10) chromosome also underwent a pericentric inversion (10p12.31q31.1). As parental chromosomes were normal, the child's karyotype was interpreted as : 46,XX,der(10)del(q26.3)dup(q26.2q25.3)dup(pter)inv(10)(p12.31q21.1) *de novo*.

In summary we report on a dysmorphic child carrying a *de novo* inverted duplication associated with a deletion of the 10qtel. From previous publications one can correlate the child's phenotype to the inverted duplication of the 10q25.3q26.2 region.

Finally as 10ptel material replaced 10qtel material, we propose that a transient ring chromosome 10 was formed to stabilize 10qtel.

#### P03.122

#### Investigation of cytogenetic causes of congenital heart disease in Pediatric Cardiology Clinic Tg Mures, Romania

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Objective: The aim of this study was to investigate chromosomal abnormalities in children with congenital heart disease (CHD) from the Pediatric Cardiology Clinic Tg Mures, Romania.

Material and method: 70 children with CHD were included in this study over a period of 2 years (2007-2009). The study included children with a diagnosis of congenital heart disease confirmed by postnatal echocardiography. In cultured lymphocytes obtained from peripheral blood, the chromosomes were stained by the GTG banding technique. Chromosome analysis was performed following ISCN guidelines.

Results: Of the 70 cases studied, 42 (60%) patients revealed obvi-

ous chromosomal abnormalities; the most part of them have involved autosomes (eg. trisomy 21, trisomy 18, Cri du Chat syndrome, pericentric inversion of chromosome 9) while only a few involved sex chromosomes (eg. Turner syndrome; polisomy X). Several cases have been diagnosed with various genetic syndromes: Williams syndrome, DiGeorge syndrome, Cornelia de Lange syndrome, Saethre-Chotzen syndrome, Klippel Feil syndrome, based on the clinical features. 28 patients with congenital heart diseases and different dysmorphic features / developmental delay / multiple congenital anomalies revealed no numerical or structural chromosomal abnormalities.

Conclusion: Genetic abnormalities are an important cause of congenital heart disease in children. Cytogenetic analysis should be the first step in the protocol of investigation in all children with congenital heart disease and multiple anomalies.

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#### P03.123

#### Interstitial de novo deletion, del(4)(p15.2p16.2), indentified in a case with non-syndromic mental retardation

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Interstitial and terminal deletion of chromosome 4p have been previously described in association with variable phenotypes. The most well defined condition is Wolf Hirschhorn syndrome (WHS) generated by 4p16.3 deletions. More proximal deletion, without WHS phenotype, but associated with varying degrees of mental retardation, were also reported.

We report on a 2 year-old girl with dysmorphic features (but lacking the facial characteristics of WHS), moderate mental retardation, spastic tetraparesis and congenital heart malformation, exhibiting an interstitial deletion 4p15.2-16.2.

GTG banding, fluorescent *in situ* hybridization (FISH) and array-based comparative genomic hybridization (BAC array-CGH) were performed. Cytogenetic slides were obtained from peripheral blood cultures, by standard protocols. For FISH investigations painting probes WC4 (Kreatech) and BAC probes (RP11-338K13, RP11-472B18) were used. BAC array-CGH using CytoChip was applied on DNA extracted from peripheral blood with Wizard Genomic DNA Purification Kit (Promega).

Classical cytogenetic analysis revealed an interstitial 4p deletion. Painting probes for chromosome 4 excluded rearrangements involving others chromosomes. BAC array-CGH was applied for a precise delineation of the deleted region. Proximal and distal breakpoints were established at 4p15.2 and 4p16.2 respectively. Array-CGH result were further confirmed by FISH with BAC RP11-338K13 and RP11-472B18 probes. Parental karyotypes were normal.

To our knowledge, an association of the described phenotype and del(4)(p15.2p16.2) has not been described so far. Our results also strongly support the need for use of various cytogenetic and advanced molecular techniques, for a correct phenotype/genotype correlation.

#### P03.124

#### Double aneuploidy mosaicism in one Romanian male case

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The co-occurrence of two numerical

chromosomal abnormalities in same individual is relatively rare. The purpose of this communication is to report another case with double aneuploidy discovered as the result of the evaluation of an infant for possible Down syndrome.

We present a male neonate, who was born to a 30 year old gravida two parity one mother, at 36 weeks of gestation, by cesarean section. The pregnancy was uneventful. At birth, the Apgar score was good. The patient's birth weight was 2750g (percentile 10), body length 49cm

(percentile 25). He had a healthy brother.

The baby had typical clinical features of trisomy 21, epispadias and bilateral syndactyly of 4<sup>th</sup>-5<sup>th</sup> toes. The patient didn't has conditions like thyroid, digestive and cardiac congenital disease.

Chromosome study from peripheral lymphocytes using GTG-banding showed mosaicism 47,XY,+21[70]/48,XXY,+21[30] analyzed in 100 metaphases. Molecular analyses were used to verify the cytogenetic result. This Down syndrome case with a rare cytogenetic abnormality as a double aneuploidy demonstrated typical Down syndrome manifestation along with additional features.

Theories concerning the origin of mixed autosomal-gonosomal trisomies are also discussed.

#### P03.125

#### A de novo double translocations 3;14 and 6;20 in a patient with mental retardation and microcephaly

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The presence of two independent apparently balanced reciprocal translocations in one person is extremely rare.

Here we report a patient with developmental delay and microcephaly who was found to have a karyotype with two apparently balanced de novo reciprocal translocations. Chromosomal rearrangements involving four chromosomes and four breakpoints. The proband was a 3-month-old male who was the product of the first multiple pregnancy (twins) of 22-year-old mother and a 28- year-old father. The parents were healthy and no consanguineous. The patient was referred to clinical geneticist for developmental retardation when he was 3 months old. His phenotypic findings included microcephaly, small forehead, closed fontanelles, relatively log ears.

Blood cultures were harvested by standard methods using thymidine block to achieve mitotic synchronization. Cytogenetic analysis was performed from GTG banded metaphases. Chromosomal analysis of peripheral blood lymphocytes revealed a karyotype of 46,XY,t(3;14)(q12;q11.2),t(6;20)(q21?p11.2) in all cells. Cytogenetic study of the proband's parents and twin sister showed normal karyotypes.

Developmental delay and microcephaly are probably due to small deletions or gene disruption at one or more breakpoints.

#### P03.126

#### Identification of supernumerary marker chromosome 15

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Among cases with supernumerary marker chromosomes the majority represents the derivatives of chromosome 15. More frequently these derivatives are presented with inv dup (15) that cause the tetrasomy of 15q. Less information is published concerning partial trisomy of 15q. In this study we report identification of a marker chromosome in a female patient, who was brought to clinical attention because of epilepsy at age 8 months. She did not have any other clinical features. Reexamination at the age of 1.8 years the patient showed psychomotor retardation. She could not sit and stand without assistance. Apart from left eye strabismus she did not have substantial dysmorphic characteristics. Classical cytogenetic analysis revealed presence of a supernumerary marker chromosome. Due to the clinical presentation the marker chromosome was suspected to be derived from chromosome 15. WCP-FISH confirmed that the marker chromosome was derived from chromosome 15.

We characterized the marker chromosome further with FISH analysis using BAC clones mapping to chromosome bands 15q15.2; 15q21.1; 15q21.2. The results suggested that the BAC clone RP11-626N18 was present on the derivative chromosome, while the BAC clones RP11-626N18 and RP11-235L4 (mapping to bands 15q21.1 and 15q21.2, respectively) were deleted. We discuss the symptoms of our case comparing with other cases with partial trisomy 15q to clarify the minimal region responsible for these symptoms developing.

For further characterization of the derivative chromosome we will apply array-CGH analysis. This may help identification of genes, dosage abnormalities of which may result in neurological and developmental disorders.

#### P03.127

##### Report of a familial inversion

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A couple was referred to us for chromosomal analysis because they had a child with growth delay.

Lymphocyte cultures from the patients were set up in RPMI1640 supplemented with 20% FBS. High resolution chromosome banding was performed.

In all cells analyzed an inversion on the p arm of chromosome (1) of both husband and wife was detected. The karyotypes were assessed as 46,XX,inv(1)(p31;p34.3) and 46,XY,inv(1)(p31;p34.3).

Therefore chromosome analysis was recommended for their affected child. Interestingly the same inversion was found for the affected child, and indeed one of the sisters of this male patient had the same inversion.

The phenotypic abnormalities presented in this child could be caused by the possible deletion of the important genes located at the breakpoint regions or it could just be a coincidence.

Chromosome study for all the siblings of this couple was recommended. In addition prenatal diagnosis for the future pregnancies of the all the carriers of this inversion was recommended.

#### P03.128

##### Unusual Cases of Trisomy 9p and Tetrasomy 9p Detected Postnatally

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Isochromosomes of autosomes are relatively rare chromosomal aberrations. Isochromosome formation results when one arm of a chromosome is lost and the remaining arm is duplicated. An isochromosome has morphologically identical genetic information in both arms. The clinical impact on patient depends on the type of the chromosome and the length of the duplicated segment. Supernumerary isochromosomes may result in autosomal trisomy or much rarely in tetrasomy which has been described only in a limited number of chromosomes.

We describe two cases of aneuploidy 9p both of which involved iso-chromosome 9p.

The first case is a trisomy 9p (Rethore syndrom) in a form of isochromosome 9p detected in a child together with translocation of 9q to chromosome 11 confirmed by FISH and CGH.

The second case describes newborn with severe malformations. Postnatal examination revealed supernumerary chromosome 9 determined by FISH as isodicentric chromosome 9p.

#### P03.129

##### Jacobsen syndrome : about three Tunisian cases

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Jacobsen syndrome is caused by terminal deletions of the long arm of chromosome 11, typically in sub-band 11q23.3 with deletions extending to the telomere.

The phenotype severity depends on the deletion shape which leads to several dysmorphic features, heart defects and Paris-Trousseau syndrome.

The first case was a 4-years old boy with dysmorphic features, cardiac defect and language delay. The second case was a two-and-a-half year old boy who was referred to our consultation for dysmorphic features, language delay, ventricular septal defect and undescended testes. The third case was an eight-month-old boy, he was referred for malformatif syndrome, he had oesophagus atresie, cardiac defect and clinodactyly of fifth fingers.

All patients have dysmorphic features compatible with Jacobsen syn-

drome.

The three patients have cardiac defect, the first case have a minor form of Ebstein disease, the second one has ventricular septal defect, the third one has auricular and ventricular septal defect and pulmonary stenosis.

None of our patients has thrombocytopenia.

All the deletions included band q23.3 of the long arm of chromosome 11 and are de novo.

The 11q terminal deletion disorder appear to be a contiguous deletion gene disorder. With molecular cytogenetic we will better delineate the critical regions and precise the correlation genotype phenotype and the identification of candidate causing genes.

#### P03.130

##### Variable phenotype of 18p monosomy in two patients

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Monosomy 18p ( de Grouchy Syndrome 1), is a rare disorder. The incidence is around 1:50,000 live born infants. The phenotype includes mild to severe mental retardation, speech delay and short stature. Various phenotypic expressions have been attributed to the deleted regions on the short arm of chromosome 18.

We describe two patients with 18p monosomy who demonstrate that hemizygosity of the genes located on the short arm of chromosome 18 cannot predict similar phenotype. Rather, multiple genetic and environmental factors contribute most probably, to the spectrum phenotype. Patient 1 is a 16 year old girl from Ethiopian origin who was born at term to non-consanguineous healthy parents. She presented to our clinic because of severe mental retardation, poor speech and communication abilities. She has short stature and short webbed neck. No other remarkable dysmorphic features were noticed.

Patient 2, is a four years old girl born prematurely at 26 weeks gestation, to healthy unrelated parents. She presented with short stature, mild mental retardation, speech delay and remarkable nasal-voice. Karyotype analysis in both cases revealed deletion of the short arm of chromosome 18, with patient 2 having complete deletion while patient 1 has near complete deletion.

These two patients demonstrate the complexity of the factors which determine the final phenotype of monosomy 18p. Genetic counseling should take into consideration the variable expression of this deletion syndrome and the importance of comprehensive medical and educational assessment, before a definite prognosis is determined.

#### P03.131

##### *Pachygyria and polymicrogyria, crano-facial dysmorphism and atrio-ventricular canal resulting from a duplication of the proximal region of chromosome 11 (p11.2)*

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Clinical abnormalities included unusual cranio-facial features. He had a microcephaly, a plagiocephaly and a small forehead, facial asymmetry with a dysplastic right ear, upslanted palpebral fissures, ptosis of the right eyelid, strabismus, long philtrum, congenital hypoplasia of the left depressor anguli oris muscle, big ears, micrognathia and a high arched palate. Physical examination revealed a continuous cardiac thrill. A large ductus arteriosus with an auricular septal defect were detected at ultrasound examination.

Congestive cardiac failure led to surgical treatment at 3 months of age. Microcephaly was detected at 8 months, and a facial asymmetry became obvious at that time. Ophthalmological examination and audiogram were normal.

His early development was severely delayed and recurrent seizures developed during the first year of life. Cerebral MRI examination showed bilateral opercular dysplasia predominant on the right side and gyration abnormalities with pachygyria and polymicrogyria.

Cytogenetic investigation from blood and skin samples demonstrated a chromosomal mosaicism, with a dicentric chromosome 11 seen with both G- and R-banding (25/25 abnormal cells on blood, 8/10 on skin) (Fig.3a). C-banding demonstrated the presence of 2 centromeres, with a small interposed euchromatic extra-material. Parental karyotypes

were normal. CGH array was performed and revealed a duplication of the 11p11-q13 region. This was then confirmed by FISH. BAC-CGH array and BAC probes (FISH) allowed the determination of the precise localization of the breakpoints in our patient. Chromosome 11 genes located in this region may play a critical role in brain development.

#### P03.132

#### FISH probe selection for preimplantation genetic diagnosis in couples with reciprocal translocation

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Individuals with balanced reciprocal translocations are at risk for adverse pregnancy outcomes due to aberrant meiotic segregation. Performing PGD by interphase FISH can decrease the risk by selectively transferring only normal or balanced embryos. We show that as long as one subtelomere probe for FISH analysis is targeted at one of the two translocation segments, the second subtelomere probe can be directed to any of the remaining three subtelomeres. In combination with an appropriately selected centromeric probe, the tri-color probe set can detect all unbalanced segregants. The feasibility of using subtelomeric probes other than those for the two translocated segments has practical value; if one probe is unavailable or if the signal is suboptimal, an alternate probe can be substituted. When centromeric probe in a third color is not available, a two-hybridization protocol performed sequentially will be necessary to detect all unbalanced segregants. This is achieved by using dual-color short and long arm subtelomere probes of one chromosome followed by a second hybridization with the subtelomere probes of the other chromosome.

This general guideline cannot be applied to two types of reciprocal translocations: cases with centromere break and cases in which the breakpoint is distal to the available subtelomere probe. The interphase FISH signal pattern in such cases cannot be predicted by examining the karyotype alone. It is therefore imperative that pre-PGD interphase and metaphase FISH analyses be performed on all translocation carriers to confirm that probes selected for PGD are appropriate. Examples of such cases will also be presented.

#### P03.133

#### Cytogenetic investigation of an interstitial deletion 4q de novo and Rieger anomaly: a case report

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Interstitial deletions of the long arm of chromosome 4 involving the region 4q25-q27 are rare occurrences. The clinical features of patients carrying similar deletions include craniofacial and skeletal anomalies, malformations of the eye, cardiac abnormalities and developmental delay. Rieger Syndrome (RS) (OMIM #180500) is an autosomal dominant disorder that conditions an abnormal eye development which results in blindness from glaucoma in approximately 50% of affected individuals (gene map locus 4q25-q26). We report on a male child aged 4 presenting with development delay, attention deficit/hyperactivity, normal growth, Rieger anomaly, small conical teeth and some mild dysmorphic features. Classical karyotyping using high resolution GTG banding revealed a *de novo* 4q (q25-q27) interstitial deletion. To define the deletion breakpoints and the extent of the deletion, CGH techniques and FISH analysis using BAC DNA are in progress. The authors enhance the importance of high resolution banding for detecting subtle chromosome 4q deletions in patients with phenotypic characteristics of Rieger syndrome and compare the present case findings with previously published data.

#### P03.134

#### De novo mosaic of ring chromosome 3: a new case with growth retardation

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There are only a few published cases of ring chromosome 3. This is the first report from Iran about a girl with a *de novo* mosaic of ring chromosome 3. Our case was the first child of healthy, non-consan-

guineous parents. The maternal age was 26 years and paternal age was 33 years at delivery. There was no problem during the pregnancy. She was born with Cesarean section at full term and there were no neonatal complications. Her birth weight was 2480 g, her height was 44 cm and her OFC was 29.5 cm. Our case was referred to our department due to growth retardation. She was 3.5 years old; her weight was 9200 g, height was 87 cm and OFC was 43 cm. Her face was triangular, with small chin, mild retrognathia and her palpebral fissures were slanted upward and outward. There was telecanthus, small alae nasi with a full nasal tip, and normally positioned, simple ears. The first two toes were widely spaced bilaterally. Her eyes, hearing, talking and IQ were normal. Karyotyping was performed on her peripheral blood. Heparinized blood samples were cultured, harvested and banded according to standard methods.

Her karyotype showed:

mos46,XX,r(3)(p26 q29) [70] / 45,X,-3 [4] / 46,XX,dic r(3;3)(p26 q29;p26 q29) [1]

Chromosomal studies of her parents were normal.

#### P03.135

#### Clinical findings and cytogenetic analysis of a ring chromosome 7 in a girl referred for suspicion of Fanconi Anaemia

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Ring chromosome 7 is a rare but well documented chromosomal aberration in man. So far at least 18 cases have been reported in the literature showing a variable but distinct pattern of phenotypic characteristics in affected individuals. Besides others, skin findings as pigmented naevi are especially frequent.

We report on a girl with mosaicism of a *de novo* ring chromosome 7. She presented for bycytopenia and was suspected for Fanconi Anemia. The main clinical features were growth failure, cafe-au lait spots and multiple pigmented naevi. Psychomotor development was normal and no major malformations were present. Chromosome analysis after R banding and FISH showed a big ring chromosome 7 in 90% of consecutively scored metaphases (46,XX,r(7)/45,XX,-7/46,XX). The medullar karyotype showed a monosomy 7.

We reviewed previously reported patients with ring chromosome 7 in an attempt to establish genotype-phenotype correlations, which are particularly important for genetic counselling and clinical genetics. Our patient may represent the first case of ring chromosome 7 with haematological manifestation.

#### P03.136

#### Clinical, cytogenetic and molecular characterization of ring chromosome 9 formation due to inverted duplication and terminal deletion

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Ring chromosome 9 is a rare chromosome aberration associated with variable phenotype that may include growth and psychomotor retardation, microcephaly, dysmorphic facial features, heart malformation, ambiguous genitalia, limb and skeletal defects. The majority of ring (9) cases arise from deletions of the chromosome with breakpoint positions between 9p22-9p24 and 9q33-q34, followed by the fusion of the ends of terminal segments. Very rarely other structural aberrations are involved. Here we describe a XY sex-reversed patient carrying ring chromosome 9 with additional material on 9p. High resolution banding suggested the presence of a duplication of band p23. Fluorescent *in situ* hybridization (FISH) analysis with whole chromosome painting probe for chromosome 9 excluded an insertion or a translocation from other chromosomes. The analysis with TelVision 9p and 9q probes identified the subtelomere - specific sequences on 9q but failed to detect a hybridization signal on 9p. The breakpoint positions and the size and location of duplication were further analyzed by molecular techniques using microsatellite DNA markers and multiplex ligation dependent probe amplification (MLPA). The karyotype was designated as 46,XY,r(9)(p24;q34.3)inv dup(9)(p24p22)mat. From 24 cases of ring (9) reported so far, there is only one case which included distal

9p duplication. This case highlights the importance of using combined molecular and cytogenetic techniques for accurate characterization of rare chromosomal rearrangements in order to make possible genotype-phenotype correlations and to understand the genetic mechanisms involved.

#### P03.137

#### Clinical characteristics of syndrome 47, XY + 18 AND 47, XX + 18 / 46, XX

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In Center for genetics during the last 10 years we had three cases of Sy Edwards, two boys and a girl.

Boys had trisomy 18., while a girl had mosaic type of trisomy 18; 47, XX + 18/ 46, XX. All died within first two months of life. By comparing the clinical characteristics of these three patients, we evaluated the intensity of characteristic symptoms and their influence on overall survival.

Phenotype did not vary significantly, all of them had typical hand position with 2. over 3. and 5. over 4. finger; rest of characteristics were identical at the boys. Girl with mosaic type of this trisomy did not have : micrognathia, irregular formed ears and insert nose base.

#### P03.138

#### A male with balanced reciprocal translocation t(5;11)(q32;q24.2) and situs inversus: case report

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Situs inversus is a condition in which the organs of the chest and abdomen are arranged in a perfect mirror image reversal of the normal positioning. The condition is in about 1 in 8,500 people. Although the mechanism that causes the heart loop to go left is not fully understood, at least one gene has been identified to have a role in this process. However, it is thought that many factors may be involved in causing situs inversus. The case was 35 years age and single person. In the imaging studies, his heart was on the right (dextrocardia), his liver was on the left, and his spleen was on the right. Cytogenetic study showed that man carried balanced reciprocal translocation: 46,XY, t(5;11)(q32;q24.2) in the man. He had no dysmorphism, but he had chronic gastritis and chronic esophageal acid reflux. Here we report a case with balanced reciprocal translocation and situs inversus.

#### P03.139

#### Confirmation of the assignment of Steinfeld syndrome to the long arm of the chromosome 13.

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Steinfeld syndrome is a rare entity described in only seven patients. It is characterized by multiple congenital anomalies namely holoprosencephaly, limb defects including thumb agenesis of the four extremities, dysmorphic signs (hypotelorism, microphthalmia, cleft lip/palate), and variable kidney and heart malformations. Differential diagnosis is represented by Garcia-Lurie syndrome also named XK aprosencephaly. Molecular bases of Steinfeld syndrome remain unknown but a pure 13q31.1-13qter deletion has been described in one foetus presenting with this disorder. The authors discuss on a possible contiguous gene deletion encompassing the ZIC2 (involved in isolated cases of holoprosencephaly), GPC5 and EFNB2 genes, and speculate on the possible involvement of these genes in the phenotype.

Here, we report on a foetus with clinical and radiological features that

full fit the diagnostic criteria for Steinfeld syndrome. Chromosomal studies reveal an unbalanced chromosomal anomaly inherited from the mother resulting in a 13q22-13qter deletion and a partial 1q32-1qter trisomy. This result confirms the assignment of Steinfeld syndrome to the long arm of the chromosome 13. FISH study using a specific probe shows a deletion of the ZIC2 gene. High resolution SNP array study (Affymetrix SNP array 6.0) is in progress to determine the exact size of the deletion in an attempt to discuss the molecular assignment of the genes involved in Steinfeld syndrome.

#### P03.140

#### Tetrasomy 9p: case report of a child with mild phenotype

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#### Background

Tetrasomy 9p is a rare dysmorphic syndrome with approximately 40 cases described to date, both mosaic and non-mosaic cases. We report a 3 years old child who has a mosaic isochromosome 9p detected postnatally. Clinical findings and cytogenetic results are presented and compared to reports of mosaic patients previously published.

#### Case Report

Second son of non-consanguineous healthy parents, with an irrelevant family history. The mother was 39 years-old at the date of the birth. No foetal anomalies were detected by repeated ultrasounds. Physical examination at birth revealed normal somatometry and cleft lip. Growth retardation, global mild psychomotor delay, hydronephrosis and hydrocele were noted later. At a Medical Genetics consultation facial dysmorphic features were observed. Height and weight under the 5th centile, microcephaly, and small hands were confirmed.

#### Cytogenetics Analysis

GTG-banded chromosome study from lymphocyte cultures of the patient revealed mos 47,XY, +i(9)(pter->p10::p10->pter)[25]/46,XY[5]. This supernumerary structurally anomalous chromosome contains a mirror duplication of the short arm of chromosome 9, with one centromere. Parental karyotypes were normal.

#### Discussion

Most secondary isochromosome formation originates from primary trisomy 9 due to maternal meiosis II nondisjunction. Isochromosome for the short arm is often found in mosaicism, since it results of a telocentric chromosome with centromere instability. Mosaicism of tetrasomy 9p presents genetic counselling problems, especially if it is de novo and diagnosed prenatally. The extent of mosaicism does not allow predicting severity of phenotype, but mosaic cases tend to have increased probability of survival compared to non-mosaic cases.

#### P03.141

#### Two new cases of mosaic tetrasomy 9p with moderate craniofacial dysmorphism and mild mental retardation

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Tetrasomy 9p is a rare chromosomal aberration leading to a syndrome resembling to trisomy 9p with additional clinical features and more severe phenotype. The recurrent breakpoints have been defined with conventional cytogenetic (9p12, 9q12; and 9q13). To date, only about 45 cases have been described. We review here two new cases of mosaic tetrasomy 9p, whose rearrangements have been further investigated by CGH-array (Bac clone 4400 Cytochip Perkin Elmer). The first one is a five-year-old boy presenting a typical phenotype with mental delay and facial dysmorphies, and subnormal mental development. The second one is a fifteen-year-old girl with moderate facial dysmorphies and no mental retardation. We have mapped the breakpoints in both cases and have compared the molecular data of our patients with published. We discuss genotype-phenotype correlation in the light of the precise breakpoints of our patients.

## P03.142

**A novel Reciprocal Translocation t(X;7) in a child with Development Delay**

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Here we report a novel translocation with breakpoints never before reported. A 5 year old girl was referred to our laboratory because of developmental delay.

Lymphocyte cultures from the patients were set up in RPMI1640 supplemented with 20% FBS. High resolution chromosome banding was performed.

Chromosome study revealed an apparently balanced translocation between chromosome X and 7. The karyotype was accessed as 46,XX,t(X;7)(q13;q32). It is possible that genes disrupted by the translocation breakpoints contribute the patient's phenotype.

## P03.143

**Variegated silencing of a large Xq region in a case of balanced X;2 translocation**

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Transcriptional silencing of X chromosome starts in the early embryogenesis of female mammals and randomly inactivates either the maternally or the paternally derived X chromosome, which thereafter shows a late replicating behavior. We studied the X inactivation pattern in a case of reciprocal balanced translocation: 46,XX,t(X;2)(Xpter->Xq23::2q35->2qter;2pter->2q34::Xq24->Xqter)de novo, presenting with a phenotype suggestive of hypomelanosis of Ito: mild mental retardation, short stature, obesity, hypo-pigmented cutaneous patches, facial dysmorphisms, myopia, hemi-hypertrophy of limbs, brachydactyly.

CGH by Affymetrix SNP array 6.0 excluded microdeletions, microduplications and loss of heterozygosity at the breakpoints. Methylation analysis at the androgen receptor locus showed completely skewed X inactivation in the proband lymphocytes. BrdU immunostaining assay, combined with in situ hybridization using whole chromosome 2 painting, demonstrated that the normal X chromosome was late replicating in 100% of the metaphases from lymphocytes, thus excluding auto-some inactivation. The same analysis, performed in skin fibroblasts, showed a late replication also in part of the Xq region translocated to chromosome 2q, in 60% of the metaphases, suggesting gene silencing in this region. Analysis of histone H3 lysine methylation confirmed the partial inactivation of the translocated Xq region in fibroblasts. Quantitative RT-PCR demonstrated downregulation of some genes mapping to Xq24qter in the proband fibroblasts.

As the altered phenotype can be ascribed neither to chromosome microdeletions/microduplications nor to inactivation of the translocated chromosome 2 region, we hypothesize that a mosaic functional nullisomy of genes mapping to Xq24qter, through a position-effect variegation mechanism, might be responsible for the phenotypic anomalies of the proband.

## P03.144

**Trisomy 8 mosaicism syndrome in 2 children from Bulgaria**

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Mosaic Warkany syndrome 2 or Trisomy 8 mosaicism syndrome/T8mS/ is a rare disorder. Over 75 cases have been reported. The clinical features are extremely variable. The authors identified 2 clinical cases with Warkany syndrome 2.

The first clinical case is 9 year's boy with Gipsy origin, born from third normal pregnancy. After this pregnancy the mother had 7 miscarriages. The clinical picture included dysmorphic facies, microretrognathia, microcephaly, many frenulums of the tongue and alveolar ridge, thoracic deformity, camptodactyly, excavated nails, hypertrichosis, mental retardation with aggressive and autoaggressive behaviour, recurrent bronchitis and asthma. The CAT showed internal hydrocephaly. The chromosome analysis in peripheral venous blood revealed trisomy 8p47, XY+8/46, XY.

The second clinical case is 9 year's old boy with Bulgarian-Turkish origin, born from first pregnancy with vacuum extraction. He presented

with mental and speech retardation, dysmorphic facies with strabismus, ptosis of left eye lid, upturned nose, thick and downturned lower lip, alopecia areata, low-set ears with incisure of the helix, high palate, camptodactyly and contracture of the fingers, kyphosis, recurrent bronchitis and asthma in early childhood, vitiligo at 7 year's old. The EEG was normal. The CAT showed internal hydrocephaly and agenesis of corpus callosum. The chromosomal analysis in peripheral venous blood revealed: 47,XY+8/46,XY. Discussion of the possible etiology and clinical results will be presented.

## P03.145

**De novo unbalanced translocations: how many of them have a post-zygotic origin?**

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Post-zygotic formation of translocations is considered a rare event in the absence of mosaicism. We have analyzed with array-CGH and microsatellites analysis of the trios eighteen de novo unbalanced translocations. In four cases, we demonstrated that the derivative chromosome was not deleted as expected but contained a distal deletion associated to a contiguous inverted duplication (inv-dup-del) on which the second chromosome segment involved in the translocation had been transposed. In the remaining thirteen cases the derivative chromosome was deleted as expected but in three cases the deleted and duplicated portions have different origin. Altogether, these findings suggest that at least some de novo unbalanced translocations derive from a dicentric chromosome. Asymmetric breakage of such a chromosome would result in the formation of an inv-dup-del chromosome and a simply deleted one and both can be healed by telomere capture from another chromosome either with different (50% probability) or the same (50% probability) parental origin. The dicentric chromosome can be formed, by NAHR and NHEJ, during gametogenesis or early embryogenesis. Mosaic situation with two or more cells line with different derivatives, each one originating from the same chromosome demonstrated the existence of a dicentric chromosome in the zygote. Its instability should lead during early embryogenesis to different breakages leading to different cell lines. The most viable one(s) will survive to term. This same mechanism of origin has been suggested also in cases of inv-dup-del ring chromosomes. Our data demonstrate that some if not all de novo unbalanced translocations have a postzygotic origin.

## P03.146

**Segmental Uniparental Disomy of short and long arm of Chromosome 18, result of parental inversion of chromosome 18**

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Here we report a case of familial pericentric inversion of chromosome 18, inv(18)(p11.2q21.3). The parents are first cousins and have identical karyotypes: inv(18)(p11.2q21.3).

Their first child's chromosomal study revealed 46,XY,rec(18)dup(18q)inv(18) (p11.2q21.3). He had mild dysmorphic features, in the absence of mental and developmental retardation. Prenatal diagnosis was performed for the second pregnancy.

Chromosomal study revealed 46,XX,rec(18)dup(18q)inv(18)(p11.2q21.3), rec(18)dup(18p)inv(18)(p11.2q21.3) detected on normal karyotype. FISH study using 18q and 18p subtelomeric probes showed two signals for 18p subtelomeric region on one chromosome 18 and two signals for 18q subtelomeric region on the other chromosome 18. Therefore chromosomal findings in karyotype were confirmed and even though the product is a balanced karyotype, the proband has uniparental disomy for the 18q21.3> region and pter>p11.2 region. The fetus was chromosomally balanced yet our concern was the possibility of imprinted gene or genes in the uniparental disomy segments. To our knowledge complete uniparental disomy of chromosome 18 has not previously been reported and there is only one report of segmental uniparental disomy of chromosome 18. Therefore genetic counseling was difficult for this couple. The couple decided to keep the child. The infant was born and is now a 1-year-old apparently healthy girl. This study supports the lack of association of uniparental disomy for these

regions with mental /developmental retardation or any dysmorphic features.

Genetic counseling issues for the family, particularly this child is important.

This child will not have chromosomal balanced gametes, unless "correction" of the recombinant chromosome occurs at meiosis due to crossover.

#### P03.147

##### Paternal uniparental isodisomy for chromosome 14 in a child with normal karyotype, resulting from malsegregation of maternal Robertsonian translocation.

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Paternal uniparental disomy for chromosome 14 (patUPD14) is a rare abnormality associated with a recognizable phenotype of small thorax, hypoplastic ribs, short limbs, abdominal wall defects and characteristic facies. Most of the patUPD14 cases have been ascertained because of an abnormal phenotype. UPD14 can result from trisomy or monosomy rescue during fetal development. Some cases are associated with a familial Robertsonian translocation (ROB), where the affected children were shown to carry the balanced translocation.

We report an 8 month old girl diagnosed with patUPD14 and normal karyotype 46,XX. Polyhydramnios, short limbs and small thorax were detected prenatally. Post natal clinical features included a narrow bell-shaped chest, short limbs, contractures of the thumbs, blepharophimosis, congenital heart defect and dysmorphic features (deep-set eyes, full cheeks, vertical chin crease and short neck). Molecular analysis confirmed complete paternal isodisomy of chromosome 14. Parental chromosome analyses showed that the mother is a carrier of der(13;14)(q10;q10). The proposed mechanism leading to patUPD14 in this patient is the duplication of paternal chromosome 14 in a monosomic conceptus resulting from maternal meiosis I nondisjunction and fertilization of a nullisomic gamete.

The majority of published UPD14 cases with normal karyotypes were not followed with parental karyotype analysis. As der(13;14) is the most common translocation in humans, we propose that chromosome analysis in parents of UPD14 patients with normal karyotype should be considered to better determine the frequency of ROB in these families.

#### P03.148

##### Down syndrome and Hirschsprung's disease

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Down's syndrome (DS) is the most common chromosomal abnormality associated with Hirschsprung's disease (HD) and patients have a worse outcome than HD alone. Incidence of DS in HD is ranging between 2% to 15%. In six years period fourteen, 12 boys and 2 girls, patients with the diagnostic of HD were admitted to our hospital. Patient's records were reviewed for data regarding age, sex, length of the aganglionosis segment, complications and functional outcomes. Age at initial presentation varied from 1 day to 7 years, mean 4, 5 years. Mean hospitalization period was 30days/ patient. Two HD patients had also DS. Patients with DS had significantly more comorbidities. Both DS patient had short segment aganglionosis. Overall there were nine patients with short segment aganglionosis, four patients had a long segment of aganglionosis and one total colonic aganglionosis .The procedure of choice in our clinic is two stages opened Duhamel pull-trough. Complete two stages Duhamel pull-trough was performed in 10 patients and four underwent temporary diverting colostomy. Two patients died from severe enterocolitis and septic shock. In DS patient's postoperative course was uneventful suggesting that DS has minimal influence on immediate surgical outcome. Long term follow up in the DS patient's revealed higher incidence of constipation, but there is improvement with age.

#### P03.149

##### Acute lymphocytic leukaemia in a child with a Beckwith-Wiedemann syndrome harbouring a *CDKN1C* germ line mutation

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Beckwith-Wiedemann syndrome (BWS) is a rare overgrowth syndrome associated with an increased risk in childhood tumours. The phenotypic variability in BWS reflects its genetic heterogeneity. This syndrome is a multigenic disorder caused by dysregulation of imprinted growth regulatory genes in the 11p15.5 region. Accordingly, epigenetic alterations, *CDKN1C* (*p57<sup>KIP2</sup>*) gene mutations, 11p15 paternal uniparental disomy and 11p15 structural chromosomal abnormalities have been described in BWS. The most commonly tumours reported in this syndrome are tumours of embryologic origin such as Wilms tumours, hepatoblastomas, neuroblastomas, rhabdomyosarcomas and adrenocortical carcinomas.

Here, we report the case of a 10 years old patient diagnosed with BWS, who developed an acute lymphoblastic T leukaemia. Lymphoblastic T leukaemia is rarer in children compared to the B-type. Molecular genetic analysis demonstrated a heterozygous *CDKN1C* (*p57<sup>KIP2</sup>*) deleterious mutation of maternal origin. To our knowledge it is the first report of an acute lymphoblastic leukaemia of T-type in a child with BWS. Based on this presentation, we discuss the possibility of a link between BWS and leukemia via one of few known negative regulator of hematopoiesis, the transforming growth factor  $\beta$  pathway, depending upon the up-regulation of the *CDKN1C* (*p57<sup>KIP2</sup>*).

#### P03.150

##### Rapid detection of methylation change in Beckwith Wiedemann syndrome and Russell-Silver syndrome using methylation-sensitive high resolution melting

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Background Beckwith-Wiedemann syndrome(BWS) and Russell-Silver syndrome (RSS) are growth disorders with opposing epigenetic mutations. Molecular diagnosis of BWS involved hypomethylation in KvDMR or hypermethylation in H19 gene on chromosome 11p15.5; while the RSS were resulted from hypomethylation in H19 gene or hypermethylation in MEST gene on chromosome 7q32.

Methods The methylation-sensitive high resolution melting(MS-HRM) was used to analyze methylation within the KvDMR, H19 and MEST gene. A total of 60 samples comprising normal control(40), BWS(13) and RSS(7) DNA were bisulfite-treated, analyzed and scored as hypomethylated, hypermethylated or normal. Results were compare with those derived by methylation-specific PCR(MS-PCR), methylation-sensitive multiple ligation-dependent probe amplification(MS-MLPA) or sequencing.

Results In MS-HRM, the normal control would have two melting peaks, representing the unmethylated and the methylated alleles, while the patients would have only one prominent melting peak indicating the methylation change. The results of MS-HRM were 100% consistent with that of the MS-PCR and MS-MLPA.

Conclusion The MS-HRM is a rapid, cost-effective and sensitive method for detection of the methylation changes in BWS and RSS. It may also serve a platform for other epigenetic studies.

**P03.151****Familial Fraxe Syndrome detected using MLPA technique**

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We have employed MLPA technique in order to study a family with non specific mental impairment. The term non-specific or non-syndromic X-linked mental retardation (MRX) was introduced to indicate a condition segregating in an X-linked manner in which male patients have no consistent phenotypic manifestations other than MR. Nineteen genes responsible for MRX have been identified so far. P106 MRX MLPA probemix (MRC Holland MLPA®) can be used to detect copy number changes of several genes on the X-chromosome that have been implicated in (non-specific) X-linked mental retardation.

Mother, a daughter (11 year old) and a son (3 year old) were analyzed using P106 MRX MLPA and a deletion was detected in AFF2 (FMR2) probe. Further analysis using conventional PCR techniques and sequencing confirmed that members of family were carriers of an expanded CGG region in FMR2 gene. Mother was carrier of an expanded allele with 70 CGG repeats, allele of daughter was expanded till 120 CGGs and allele of son was 135 CGGs. Son has a more severe phenotype, but both mother and daughter had a mild mental retardation. FRAXE fragile site associated mental retardation remains unique among X-linked mental retardation phenotypes due to its very mild to borderline nature (50 < IQ < 85). It is the most prevalent form of non-specific X-linked mental retardation so far delineated, with an estimated incidence of at least 1/50-100,000 males. The FRAXE site is within, or immediately adjacent to, the 5' untranslated region of the FMR2 gene.

**P03.152****Prevalence of Fragile X syndrome in mentally retarded patients from Latvia**Z. Daneberga<sup>1,2</sup>, Z. Krumina<sup>1</sup>, B. Lace<sup>1,2</sup>, D. Bauze<sup>1</sup>, N. Pronina<sup>1</sup>, R. Lugovska<sup>1</sup>;<sup>1</sup>Medical Genetic Clinic, Riga, Latvia, <sup>2</sup>Riga Stradiņš University, Riga, Latvia.

The most common form of X-linked mental retardation (XLMR) is the Fragile X mental-retardation syndrome (FXS). Mutations at *FRAXA* locus on distal Xq may cause mental impairment. Most common mutation at *FRAXA* locus is expansion of CGG triplet repeats located in the 5'-untranslated region of the *fragile X mental retardation-1* (*FMR1*) gene.

The aim of this study was to estimate the prevalence of FXS in Latvia and characterize the *FMR1* CGG-repeat structure in Latvian patients exhibiting mental retardation. The group of 374 unrelated patients with mental retardation (MR) referred from clinical geneticists was screened by PCR for a normal allele. The final diagnosis of FXS has been confirmed by Southern blotting. DDX548-FRAXAC1-FRAXAC2-ATL1 haplotype for FXS patients were estimated. DNA sequencing for the estimation of AGG inserts structure for gray zone (35-50 repeats) alleles was used.

10 affected patients were detected (detection rate 2.67%). Calculated prevalence of *FMR1* full mutation is 1:6173 for male in general Latvian population. After active cascade testing in 6 FXS families 6 female permutation carriers, 3 females with full mutation and 4 affected males were found. The highest incidence among FXS patients for haplotype 4-3-4-G was found. The prevalence of 29, 30 and 31 CGG repeats for normal alleles were detected.

**P03.153*****FMR1* gene stability: distribution of premutation and intermediate alleles in five basque valleys**I. Arrieta Saez<sup>1</sup>, M. Telez<sup>1</sup>, I. Huerta<sup>1</sup>, P. Flores<sup>2</sup>, B. Criado<sup>3</sup>, J. Ramirez<sup>1</sup>, M. Barasoain<sup>1</sup>, M. Hernández<sup>1</sup>, A. González<sup>4</sup>;<sup>1</sup>Department of Genetics, Faculty of Science and Technology, University of the Basque Country, Bilbao, Spain, <sup>2</sup>Department of Nursing, School of Nursing, University of the Basque Country, Bilbao, Spain, <sup>3</sup>High School Da Maia, CESPU, Porto, Portugal, <sup>4</sup>Department of Internal Medicine, Faculty of Medicine, University of the Basque Country, Bilbao, Spain.

Fragile X Syndrome (FXS) is the most common form of inherited mental retardation. The molecular basis is usually the unstable expansion of a CGG repeat in the *FMR1* gene. The CGG sequence is polymorphic with respect to size and purity of the repeat. We had previously analyzed a sample of two Basque valleys (Markina and Arratia). In the present work we extend the study to another five isolated valleys

(Uribe, Gernika, Durango, Goierri and Larraun). The results showed that differences in factors implicated in CGG repeat instability (CGG repeat size, DDX548-FRAXAC1 haplotypes and AGG interspersion pattern) are present in the Basque populations analyzed.

**P03.154****Prevalence of the expanded alleles of the *FMR1* gene in blood spots from newborn males in a Spanish population**I. Fernández-Carvajal<sup>1</sup>, P. Walichiewicz<sup>2</sup>, X. Xiaosen<sup>2</sup>, R. Pan<sup>2</sup>, P. J. Hagerman<sup>3,4</sup>, M. J. Alonso<sup>1</sup>, J. J. Tellería<sup>1</sup>, A. Blanco<sup>1</sup>, F. Tassone<sup>2,5</sup>;<sup>1</sup>11.B.G.M.Unidad de Diagnóstico Genético y Perinatal. Universidad de Valladolid, Valladolid, Spain, <sup>2</sup>Department of Biochemistry and Molecular Medicine, University of California, School of Medicine, <sup>3</sup>Department of Biochemistry and Molecular Medicine, University of California, School of Medicine, Davis, CA, United States, <sup>4</sup>M.I.N.D. Institute, University of California Davis HealthSystem, Sacramento, CA, United States, <sup>5</sup>M.I.N.D. Institute, University of California Davis HealthSystem, Sacramento, CA, United States.

Fragile X syndrome is the most common inherited form of intellectual disability. The frequencies of full mutation (>200 CGG repeats) have varied widely from 1/ 2,000 to 1/ 8,000 depending on the nature of ascertainment. There remains uncertainty regarding the premutation allele frequencies due in part to lingering issues of ascertainment bias, but also to real frequency differences across ethnic and regional populations.

**Setting and Methods:** We report results of a newborn screening study of 5,267 male blood spots collected from Castilla y Leon, the Northwest region of Spain. The blood spots were screened by a rapid PCR-based method that is capable of identifying the presence of all expanded alleles categorized as intermediate alleles (45-54 CGG repeats), premutation (55-200 CGG repeats), and full mutation (>200 CGG repeats).

**Results:** The most common alleles, 29 and 30 CGG repeats, within this population accounted for approximately 38% of all. We found 199 gray-zone alleles (1 in 26; 95%CI, 1/23 - 1/30), 21 premutation alleles (1 in 251; 95%CI, 1/164 - 1/385), and 2 full mutation alleles (1 in 2,633; 95%CI, 1/714 - 1/10,000).

**Conclusions:** The frequency of premutation alleles is three times higher than the oft-quoted value of 1 in 813 from an Eastern Canadian population, and is fully consistent with the results of large-scale Israeli screening studies. Our results demonstrate that newborn screening for the presence of expanded *FMR1* alleles is an effective means for defining the distribution of expanded *FMR1* alleles in newborn populations and is suitable for large-scale newborn screening.

**P03.155****Molecular Genetic Test for X-Fragile Syndrome in 2008 IVI Assisted Reproduction Treatments.**

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Since 2007, our company, "Instituto Valenciano de Infertilidad" (IVI) has implemented the routine molecular diagnosis of X-FRAGILE Syndrome (FRAXA) into the oocyte donation program. FRAXA is one of the greatest genetic prevalence illnesses in general population. The prevalence of full-mutation males in white population is approx. 1/4,000. FRAXA is the most common cause associated to mental family inherited and represents among 15-20% of the total mental delay related to X cr. This illness has its origin in the deficiency of *FMR1* protein synthesis. The expansion of the "dynamic" and repetitive region CGG, 5' to *Fmr-1*, causes its methylation and repression of expression. According repetitions number of the CGG tri-nucleotide, this region is considered normal (<54 CGGn), premuted (55-200 CGGn) or full mutated (>200 CGGn).

We present our most recent data for these analysis, using the Abbott Molecular protocol, called Fragile X-PCR Test. During 2008 we studied 3,485 women from different cities in Spain. All these women were susceptible oocyte donors for processing of assisted reproductive treatments (ART). In conclusion, we found 17 (0,49 %) premutation carriers; 52 (1,49%) "intermediate" carriers and 1 (0,028%) possible full mutation carrier; 1/50 women were excluded from the donation program.

The knowledge of the fragile X premutation carrier condition or full mutation carrier will permit the donor to receive the appropriate genetic counsel for reproductive end. Finally, the exclusion from the oocyte

donation program of possibly "expanded" trinucleotids of this region provides greater security to the receptor patients in our processing of Assisted Reproduction.

#### P03.156

#### Fragile X syndrome screening of families with consanguineous and non-consanguineous parents in the Iranian population

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Fragile X syndrome is the most common form of inherited mental retardation (MR). It is caused by the expansion of CGG triplet repeats in the fragile X mental retardation 1 (FMR1) gene. In mentally retarded males, the frequency of fragile X syndrome is approximately 2 to 3 percent, but little is known about its proportion in mentally retarded patients from countries where parental consanguinity is common.

The objective of this study was to estimate the frequency of fragile X syndrome (FXS) in mentally retarded patients from Iran. We examined a total of 508 families with MR that had been referred to the Genetics Research Center (GRC) in Tehran. 467 of these families had at least two mentally retarded children, and in 384 families, the parents were related. Full FMR1 mutations were found in 32 of the 508 families studied (6.3%), in 19 out of 124 families with apparently unrelated parents (15.3%), and in 13 of the 384 consanguineous families (3.4%). Thus, in Iran, the relative frequency of FXS seems to be higher than in Central Europe and other Asian countries, and much higher in patients with unrelated parents. We also show that even in families with consanguineous parents, FXS has to be ruled out before assuming that familial MR is due to autosomal recessive gene defects. Molecular studies are in progress to explain the high proportion of FMR1 mutations in mentally retarded offspring of unrelated Iranian parents.

#### P03.157

#### Simultaneous analysis of FMR1 and ARX genes improves the efficiency of the fragile X screening.

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Fragile X syndrome is the most frequent form of inherited mental retardation, caused by the expansion of a CGG triplet in the *FMR1* gene. On the other hand, microduplications or expansions in *ARX* gene, leading to elongations of a polyalanine tract, are considered the second most frequent cause of X-linked mental retardation. However, the genetic screening of this gene is not routinely performed.

We conducted a prospective analysis of both genes, by the simultaneous amplification of *FMR1* exon 1 and a fragment of *ARX* gene where the polyalanine elongations cluster, in a total of 700 developmentally retarded males remitted for fragile-X screening.

We found nine unrelated cases with the fragile X syndrome and three index cases (four patients) with *ARX* elongations. Given the relative frequencies between both genes and the prevalence of the fragile-X expansions (about 1:5,000 males), the prevalence of *ARX* elongations can be estimated in 1:15,000 males.

The duplex analysis we propose offers several advantages: 1) An increased sensitivity of the fragile-X screening in about 30%, with no substantial cost increment. 2) As large fragile-X expansions cannot be detected by PCR, the amplification of another fragment with similar size, GC content and presence of repeats, serves as internal reference to differentiate mutations (expansions or deletions) from technical pitfalls. 3) Similarly, weak co-amplification of a normal *FMR1* allele can be suggestive of mosaicism with full mutation. Although rare, mosaic cases represent a limitation of any PCR-based method for the screening of the fragile-X expansion.

#### P03.158

#### FMR2 protein, whose absence causes the FRAZE associated mental retardation, is a splicing factor

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Australia.

FRAZE is a form of mild to moderate mental retardation due to the silencing of the *FMR2* gene. The cellular function of *FMR2* protein is presently unknown. By analogy with its homologue AF4, *FMR2* was supposed to have a role in transcriptional regulation, but robust evidences supporting this hypothesis are lacking. We observed that *FMR2* co-localizes with the splicing factor SC35 in nuclear speckles, the nuclear regions where splicing factors are concentrated, assembled and modified. Similarly to what was reported for splicing factors, blocking splicing or transcription leads to the accumulation of *FMR2* in enlarged, rounded speckles. *FMR2* is also localized in the nucleolus when splicing is blocked. We have recently shown that *FMR2* is able to specifically bind the G-quartet-forming RNA structure with high affinity. Remarkably, *in vivo*, in the presence of *FMR2* the ESE (Exonic Splicing Enhancer) action of the G-quartet situated in mRNA of an alternatively spliced exon of a minigene or of the putative target *FMR1* (fragile X Mental retardation & gene) appears reduced. Indeed, the absence of *FMR2* does not affect the total expression of *FMR1* mRNA but increases the expression of the isoforms containing exon 14 and encoding a protein localized in the cytoplasm. The role of *FMR2* in splicing is not exclusive to its role in transcriptional regulation, since we cannot exclude that the function of *FMR2* is modulated by the interaction with transcription factors, as, shown, for example, for SWI/SNF, a complex involved both in chromatin remodelling and regulation of alternative splicing.

#### P03.159

#### Post axial polydactily and costo-vertebral anomalies in a 31 years old woman with 1qter chromosome deletion.

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Subtelomeric deletion of long arm of chromosome 1 is described as a recognisable syndrome presenting with mental retardation, microcephaly, growth retardation, a characteristic facial appearance, corpus callosum abnormalities, cardiac, gastro-oesophageal and urogenital defects (van Bon et al., 2008).

We describe a 31 years old woman with a *de novo* 1qter deletion. We have seen her for the first time at 30 years, identified 1qter deletion by subtelomeric analysis, and characterized the size of the deletion by CGH array.

She was born to healthy unrelated parents, pregnancy was complicated with threatened abortion and growth delay (at term: weight 2.300 gr, length 48 cm, OFC 31 cm). She presented with severe hypotonia, cyanosis, cleft palate, post-axial polydactyly of left foot, bilateral clubfoot, and dysmorphic features including bulbous nasal tip, thin lips and left preauricular tag. Echocardiography revealed interventricular septal defect. Thoracic X-Rays showed dorsal vertebral clefts (D4, D5 and D6), and rib fusions (I and II, V and VI, VII and VIII at left and VIII and IX at right). Ectopic left kidney and albinoid fundus oculi were also present. Since the first months of life she had generalized seizures treated with anticonvulsivants. This is the oldest patient with 1qter deletion described to date. Her phenotype includes features not reported so far such as polydactyly and ribs fusions and confirms that vertebral anomalies, previously described in 2 patients only, are not uncommon elements of the syndrome.

#### P03.160

#### Evaluation of a case with 5p deletion syndrome

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The case is the second child of the couple who made third cousin marriage. She had a healthy elder brother. The case was born with a birth weight of 2,720 kg and a height of 52 cm. Her growth retardation was realized at the age of 8 months. The case was physically examined and a pedigree was drawn. Metaphase plaques were obtained from peripheral blood lymphocytes of the case cultured for 72 hours and evaluated after GTL banding. The findings of the case were congenital malformation, mental retardation, cat-like cry at the first 1-2 weeks after birth, SGA (small for gestational age), growth retardation, microcephaly, strabismus, micrognathia, stridor, low set ears, high arched

palate, flat broad nose root, loose abdominal muscles. In the USG performed, no abdominal abnormality and organomegaly were detected. The karyotype of the case was found to be 46,XX,del(5p) in the chromosome analysis. 5p- (Cri du Chat) Syndrome is caused by a deletion on the short arm of chromosome 5 and its incidence ranges between 1:15 000 - 1:50 000 in live births. In the cytogenetic and phenotypic investigations performed on the short arm of chromosome 5, deletions in specific size and shape were found to be related to the specific phenotypic properties. Metaphase plaques obtained from peripheral blood lymphocytes were investigated and the karyotype of the case was detected to be 46,XX,del(5p).

#### P03.161

#### Identification of Two Novel Cases with Terminal Long Arm Deletions of Chromosome 1 Displaying Microcephaly, Epileptic Seizures, Corpus Callosum Abnormalities

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Majority of the patients with chromosome 1q terminal deletions have similar clinical features such as mental retardation, hypotonia, microcephaly, seizures, corpus callosum abnormalities, congenital cardiac defects and various facial features.

Here we report two cases with overlapping deletions of the region 1q43-q44 that have been characterized by BAC array CGH (1 Mb resolution). The first patient had a 6.7-7.9 Mb interstitial deletion at 1q43-q44 chromosomal region. She had mental retardation, developmental delay, microcephaly, seizure with or without fever and dysmorphic facial features. Magnetic resonance imaging (MRI) investigation revealed hypoplasia of the corpus callosum. The second patient had two consecutive interstitial deletions of 2-4 Mb and 4-6.4 Mb, at 1q41 and 1q43-q44, respectively. He had microcephaly, febrile seizures, ventricular septal defect (VSD), hypospadias, cryptorchidism, and high palate. MRI investigation revealed dysgenesis of the corpus callosum and diffuse cerebral atrophy.

In earlier studies, serine/threonine kinase AKT3 gene at 1q44 has been suggested as a candidate for microcephaly and corpus callosum agenesis, but was excluded by mapping of the interstitial deletions involving this region. These studies suggested a new critical region including four refseq genes, namely *C1orf100*, *ADSS*, *C1orf101* and *C1orf121* for corpus callosum abnormalities at 1q44. It was deleted in our two cases. Meanwhile, when we compared the sizes of the deletions in Patient 2 and previously identified the cases with VSD and terminal 1q deletion, we refined about a 2Mb new critical region for VSD associated with 1q43 deletion.

#### P03.162

#### A case of *de novo* pure partial trisomy (6)(p22.3-pter)

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We report on a 3-year-old girl with psychomotor retardation, speech development delay, hypothyroidism, and a special pattern of cranio-facial anomalies. Karyotype analysis showed additional material of unknown origin on the short arm of chromosome 6. Molecular cytogenetic analysis, by Agilent 44K array CGH, demonstrated a *de novo* 27.9 Mb duplication from 6p22.3 to 6pter, with no obvious abnormality on the other chromosomes. Thus, our patient is characterized as being of pure partial trisomy 6p, which could be distinguished from most cases found to date. The clinical findings in this patient were compared to those in the previously reported cases. Genotype-phenotype correlation suggests further splitting in the partial 6p trisomy syndrome. The distinct facial features including prominent forehead, blepharophimosis, blepharoptosis, bulbous nose and small pointed chin, are consistently recognizable in distal 6p duplication, while severer symptoms such as feeding problems, recurrent respiratory infections, gastrointestinal anomalies, limb deformities are more likely associated with proximal 6p duplication.

#### P03.163

#### Cri du chat syndrome - clinical and genetic study of a particular case

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Cri-du-chat syndrome is a genetic disease resulting from a deletion of variable size occurring on the short arm of chromosome 5. Main clinical features are: high-pitched monochromatic cry, microcephaly, broad nasal bridge, epicanthal folds, micrognathia, abnormal dermatoglyphics and severe psychomotor and mental retardation. Cardiac, neurological and renal abnormalities may be associated.

We present a case of a 6 years old patient with "cri-du-chat" syndrome and complex abnormalities of the karyotype in order to illustrate a rare form of the disorder and to discuss the management of the patient and her family.

Anamnestic data show that the girl is the second child born to a young, unrelated, apparently healthy couple. Pregnancy was uneventful. The child was born naturally, at term, with low birth weight - 2000g. Postnatal development was severely delayed (walked at 5 years, first words at 4 years).

Clinical examination of the child (6 years old) reveals: failure to thrive, microcephaly (-5,9 SD), narrow mongoloid palpebral fissures, epicanthal folds, high-pitched voice, micrognathia, displastic ears, severe mental retardation.

Echocardiography was normal, as well as renal ultrasound. Psychological examination: IQ 29. A G band karyotyping has been performed and the result is: 45,XX,der(5)t(5;22)(5p15.1;22q.1)/46XX, der(5)t(5;22)(5p15.1;22q.1)der (22)(pter→q11.1). FISH analysis confirmed the translocation and the breakpoint on chromosome 22 (22q11.2; 22q.13). The karyotypes of the parents have been normal.

In conclusion we present a case with delayed diagnosis and complex karyotype to illustrate the importance of the karyotype and to discuss genetic counselling in this case.

#### P03.164

#### Haploinsufficiency of the gene Quaking (QKI) is associated with the 6q terminal deletion syndrome

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Several subtelomeric aberrations, especially deletions, are associated with clinically recognizable syndromes, including 1p-, 4p-, 5p-, 9p-, 9q-, 18p- and 22q-. The recent introduction of array-comparative genome hybridization in the diagnostic clinic not only enables to identify these aberrations, but also to delineate the size and locate the position of the different breakpoints. Comparison of the different sizes and breakpoints of the affected chromosomal regions permits a first correlation of the genotype with the phenotype. However, human subtelomere regions are gene-rich and it still remains difficult to assign the contribution of each individual gene within the deleted region. Recently, the *Eu-HMTase1* gene was identified as the major gene causing the 9q subtelomeric phenotype, by studying a balanced translocation involving chromosomes 9 and X.

Subtelomeric rearrangements involving chromosome 6q have been reported in a limited number of studies. Although the sizes are very variable, ranging from cytogenetically visible deletions to small submicroscopic deletions, a common recognizable phenotype associated with a 6q deletion could be distilled. The main characteristics besides mental retardation are hypotonia, seizures, brain anomalies, and specific dysmorphic features including short neck, broad nose with bulbous tip, large and low-set ears and a fish-like mouth. We report a female patient, carrying a reciprocal balanced translocation t(5;6), presenting with a clinical phenotype highly similar to the common 6q-phenotype. Breakpoint analysis using array painting revealed that the *Quaking (QKI)* gene that maps in 6q26 is disrupted, suggesting that haploinsufficiency of this gene plays a major role in the 6q- clinical phenotype.

## P03.165

**Interpretation of copy number changes of the 1p36.3 terminal region detected by high resolution oligonucleotide array CGH**E. J. Seo<sup>1</sup>, K. R. Chun<sup>1</sup>, J. O. Lee<sup>2</sup>, H. W. Yoo<sup>3</sup>, I. S. Park<sup>3</sup>;<sup>1</sup>Dept. of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Republic of Korea, <sup>2</sup>Genome Research Center for Birth defects and Genetic disorders, Asan Medical Center, Seoul, Republic of Korea, <sup>3</sup>Dept. of Pediatrics, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Republic of Korea.

Breakpoints of the 1p36 deletion syndrome have been observed between 0.5 and 10.5 Mb from the 1pter and common deleted regions are terminal 2.5-2.7 Mb. While deletions or duplications of less than 0.5 Mb from the 1pter are regarded as benign CNVs, copy number changes of more than 0.5 Mb should be assessed cautiously in factors influencing the risk. We detected various copy number changes of the 1p36.3 terminal region using Agilent 244K whole human genome CGH oligonucleotide array in 60 Korean patients with congenital anomalies. Out of 6 patients with copy number changes at 1p36.3, three showed the recurrent copy number changes of 1.56 Mb with low log2 ratio: 1 loss, and 2 gains. A patient with 1.56 Mb loss had total 5.86 Mb deletion at 1p36.3 including extended 4.3 Mb loss with high log2 ratio, which was resulted from derivative unbalanced translocation, der(1)t(1;14)(p36.3;p11.2)dn. The 1.56 Mb gain of two patients appeared to be benign CNV. Another two patients with copy number loss of high log2 ratio had 2.2 Mb simple terminal deletion inherited from an affected mother, and 2.7 Mb deletion by derivative unbalanced translocation, respectively. Remaining one patient with 2.7 Mb gain of low log2 ratio revealed 1q12-1q23.2 gain of high log2 ratio which was compatible with his marker chromosome. These findings suggest that a recurrent breakpoint of CNV could be located 1.56 Mb from the 1p telomere, and the log2 ratio should be considered when interpreting the array CGH data in constitutional disorders.

## P03.166

**Array CGH analysis of 4q terminal deletion diagnosed in a girl with mild dysmorphic features, developmental delay and no major congenital anomalies**K. Schlaude-Bartusiak<sup>1</sup>, A. Innes<sup>2</sup>, M. Chan<sup>1</sup>, M. Anderson<sup>2</sup>, J. Chernos<sup>1,2</sup>;<sup>1</sup>Cytogenetics Laboratory, Alberta Children's Hospital, Calgary, AB, Canada,<sup>2</sup>Department of Medical Genetics, University of Calgary, Calgary, AB, Canada.

The 4q deletion syndrome, comprising all cytogenetically visible deletions (interstitial or terminal) of the long arm of chromosome 4, is a well-recognized distinctive disorder, described in more than 100 patients. Common phenotypic features of 4q- syndrome are mild dysmorphic features, mild to severe mental retardation, growth deficiency, cleft palate, limb anomalies, cardiac and genitourinary defects. A unique finding consisting of a stiff, pointed 5<sup>th</sup> finger with a hypoplastic distal phalanx and either a hooked or a volar nail is observed in two thirds of patients. Patients with large terminal deletions, with breakpoints in 4q31, are most severely affected. More distal 4q deletions involving bands 4q33 to 4q35 have been found so far in about 25 cases. These patients present less characteristic dysmorphisms and less severe mental retardation. Some authors suggest that the region 4q31-q34 is critical for most of the clinical phenotype. *HAND2* in 4q33 has been proposed as a candidate gene for the cardiac defects, and a gene involved in limb deficiencies was tentatively assigned to 4q33. It is clear that phenotypes associated with 4q deletion are grossly related to the breakpoint location and the amount of genetic material lost.

We present a 12-year old girl with mild dysmorphic features and developmental delay with learning difficulties. Karyotype analysis revealed a terminal deletion on chromosome 4q. Array CGH showed a deletion of over 17 Mb, with the breakpoint within the 4q34.2 band.

Literature review and genotype-phenotype correlations in 4q deletion syndrome will be discussed.

## P03.167

**Two new cases of pure 2q terminal deletion**

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The subtelomeric regions are gene-rich and often involved in chromosomal rearrangements. We report two patients referred to medical evaluation at the Genetic Service of SARAH Network of Rehabilitation

Hospitals-Brasília with subtelomeric chromosome 2q deletion.

Patient 1 is a female referred because of developmental delay at 17 months with epicanthal folds, asymmetric palpebral fissures, small nipples and abnormal palmar and plantar creases. She returned after 16 years, at 20 years, mild mental retardation (MR) is evident requiring support for daily life activities. Only height is below the 3<sup>rd</sup> centile. She has round face, down-slanted palpebral fissures, depressed nasal bridge, small posteriorly angulated ears, shortened forearms, brachydactyly, hallux valgus and joint hypermobility. The 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> metacarpals and metatarsals are shortened. Cytogenetic study at 550-band levels revealed a terminal *de novo* deletion with the breakpoint at 2q36, confirmed by subtelomeric probe FISH.

Patient 2 is a female referred because of MR, eczema and obesity. At 14 years, we noted up-slanting palpebral fissures, eczema involving scalp and her small hands and feet. Moderate MR, tantrums and controlled seizures were present. Cytogenetic study at 700-band levels revealed a tiny terminal *de novo* deletion at 2q37.2-37.3, confirmed by subtelomeric probe FISH.

These two cases demonstrate the clinical variability involving confirmed chromosome 2q terminal deletion. Broadening its clinical spectrum could help health professionals recognize subtle manifestations in the context of idiopathic MR. It also stresses the importance of using subtelomeric FISH studies in cases of suspected cryptic rearrangements, when investigating patients with idiopathic MR.

## P03.168

**Detection of subtelomere imbalances using MLPA**L. Hila<sup>1</sup>, Y. Chaghrani<sup>1</sup>, H. Tébourbi<sup>1</sup>, L. ben Jemaa<sup>2</sup>, H. Chaabouni<sup>2</sup>;<sup>1</sup>Laboratoire de Génétique Humaine Faculté de Médecine de Tunis, Tunis, Tunisia, <sup>2</sup>Service des Maladies Congénitales et Héréditaires EPS Charles Nicolle, Tunis, Tunisia.

Subtelomeric rearrangements significantly contribute to idiopathic mental retardation and result in several mental retardation syndromes. New molecular techniques like Multiplex Ligation dependent probe Amplification (MLPA) allow subtelomeric microduplications/microdeletions identification in approximately 5% mentally retarded patients. However most of subtelomeric defects lack a characteristic phenotype.

We analyzed Tunisian mentally retarded patients by MLPA Multiplex Ligation Probe dependent Amplification with SALSA MLPA kit P036B/MLPA kit P036D and MLPA kit P070 (which test all the subtelomeres). MLPA results for the short arm of the acrocentric chromosomes were not considered as significant as these probes hybridise to the long arm near the centromere.

DNA was extracted from blood, quantified by spectrophotometry (Nanodrop) and checked for degradation on an agarose gel, degraded DNA was not used for MLPA analysis.

Patients with known sex chromosome abnormalities were used as controls (45, XO; 47, XYY).

Relative probe signals of each probe was determined by dividing each measured peak height by the height of patient reference peak divided by the mean of height of control test peak divided by the height of control reference peak. All peaks were insured to be between 50-6000 arbitrary fluorescents units.

We found subtelomeric deletions and duplications. These results will be discussed upon the patient's clinical features to make imbalance-phenotype correlations to discern which abnormalities are disease causing or probable polymorphisms, in fact 15 subtelomeric copy number changes are reported in phenotypically normal individuals.

## P03.169

**Subtelomeric rearrangements in patients with idiopathic mental retardation**

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Mental retardation affects between 1 to 3% of the general population and is associated with several causes including environmental factors, chromosomal abnormalities and monogenic diseases. 50% of the cases have no etiologic diagnosis. Recently mental retardation was linked to subtelomeric chromosomal abnormalities.

The objective of this work is the study of patients with idiopathic mental retardation associated with dysmorphic features and/or birth defects and/or family history of mental retardation in Trás-os-Montes region - Portugal, by standard cytogenetic and FISH (Fluorescent *in situ* hy-

bridization) techniques.

In 25 peripheral blood samples were applied conventional cytogenetic techniques and FISH with the use of probes specific for the subtelomeric chromosomes regions.

The authors present the results, comparing them with those described in the literature.

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#### P03.170

##### Clinical features in 102 patients with Angelman syndrome

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**Background:** Angelman syndrome (AS) is a neurodevelopmental disorder caused by a lack of expression of the maternal copy of *UBE3A*. We are conducting a 5-year longitudinal study on the natural history of AS, to improve our understanding of the complications, morbidity and neurodevelopment in AS. We present the baseline clinical data that describes characteristics of the first 102 subjects enrolled in our study.

**Methods:** All subjects were between 5 months and 26 years old. Subjects were evaluated by detailed history, physical examination, standardized neurodevelopmental assessments, and electroencephalograms (EEG). Deletions were sized using a chromosome 15-specific comparative genomic hybridization microarray.

**Results:** The median age of the 102 subjects was 36 months (80% were between 17 months and 60 months). 74% of subjects had deletions, 15% had either imprinting defects or uniparental disomy, and 12% had *UBE3A* mutations. The most common findings were mouthng behavior in 93%, short attention span in 91%, microcephaly in 81%, sleep difficulties in 79%, ataxic or broad-based gait in 78%, fascination with water in 75%, and inappropriate laughter was observed in 62% of subjects. Clinical seizures were reported in 69% of subjects, although all EEGs were abnormal. Data on the variations in these characteristics by molecular subtype will be presented.

**Conclusions:** In children with AS, it is the neurobehavioral phenotype, rather than the dysmorphic features or seizures, that is most characteristic. The "classic" AS phenotype is more frequently observed in those with deletions or *UBE3A* mutations, and not as evident in those with UPD or imprinting defects.

#### P03.171

##### Particular forms of Prader-Willi syndrome - clinical and genetic study

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Prader-Willi syndrome (PWS) is a relatively common disorder due to abnormalities in the 15q11.2-q13 region. Major manifestations include hypotonia with poor suck and poor weight gain in infancy, early childhood-onset hyperphagia and obesity, characteristic appearance, hypogonadism, growth hormone insufficiency causing short stature, mild mental retardation and characteristic behaviour.

We present 3 particular cases of PWS recorded in the files of Iasi Medical Genetics Center in order to illustrate some special features and to discuss the diagnosis and management strategy. In all 3 cases the suspicion of diagnosis was based on actualized diagnostic criteria.

Case 1: 10 years old girl with typical neonatal hypotonia and poor weight gain in infancy followed by marked hyperphagia and obesity, acromicria and hypogonadism, but with tall stature, multiple severe allergies, severe mental retardation and seizures. Genetic defect: microdeletion identified by FISH;

Case 2: 6 years old girl with neonatal hypotonia and feeding difficulties

followed by marked obesity, but with macrocephaly, inexpressive face and severe tibia vara. Genetic defect: imprinting defect;

Case 3: 20 years old boy with typical neonatal hypotonia and poor weight gain in infancy followed by mild obesity and relatively normal appetite, acromicria (but with shortening of the 4<sup>th</sup> metacarpal) and a particular psychological profile. Genetic defect: 15q deletion identified on the karyotype.

Clinical features, diagnosis and management will be illustrated in detail. In conclusion, we present 3 particular cases of PWS in order to illustrate some special features and to discuss the diagnosis and management strategy.

#### P03.172

##### Oro-dental phenotypic spectrum of patients with PWS

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**Background.** Prader-Willi Syndrome (PWS) is a rare genetic disorder caused by genetic defects in certain regions of chromosome 15q11-13. Commonly associated characteristics of this disorder include hypotonia, obesity, mental retardation, short stature, hypogonadotropic hypogonadism, small hands and feet, facial dysmorphia, learning and behavioural difficulties, and dental abnormalities (thick viscous saliva).

**Aim.** To describe the oro-dental phenotypic spectrum of patients with PWS.

**Design.** Five PWS patients (5-22 years of age) being followed at the Emergency Children Hospital L. Turcanu, Timisoara were examined at the dental clinic of the same institution. Medical information collected included all clinical manifestation, body mass index (BMI), level of cognitive functioning, genetic investigations. Oral and radiological evaluations were performed.

**Results.** All 5 patients had caries experience, dental erosion and salivary flow rates were assessed.

**Conclusion.** The clinical implications of the dental anomalies, with genetically controlled patterns are important in establishment of early diagnosis and appropriate orthodontic care and collaboration between dentist, geneticist and pediatrician ensure a formula of a correct diagnosis and in giving an adequate therapeutic advice.

#### P03.173

##### Contributions to expanding the concept genetic-epigenetic and the correlation phenotype-genotype in Prader-Willi/Angelman syndromes

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Prader-Willi and Angelman syndromes are two distinct neurodevelopmental disorders, caused by several genetic/epigenetic mechanisms involving chromosome 15. Recent studies revealed differences in different classes of PWS/AS against previous ones. This aspect supports the requirement of extending the investigation protocols for the patients in order to acquire an early diagnosis, an accurate etiological subtype that will support an adequate genetic counseling and an efficient management of the disease. We initiated a project and we propose to identify possible factors involved in etiology of altering of the imprinting process, by applying adapted surveys for the families of the patients, including many generations. Applications of genetic and epigenetic testing relevant for subtypes of PWS/AS and the introduction of chromatin remodeling tests by chromatin immunoprecipitation assay and possibility of TOF mass spectrometry are original and complex aspects that this study has as objectives. In the first three months from the start off of this project we investigated the first four patients (1 boy and 3 girls) that were included in the study. The phenotype of the patients allowed as according to clinical criteria to set the PWS diagnose. For all patients FISH analysis was performed using Vysis Prader-Willi/Angelman Region Probe - LSI SNRPN SpectrumOrange/CEP 15 (D15Z1) SpectrumGreen/LSI PML SpectrumOrange probe. For one of the patients the deletion was positive and for the rest the result was negative. The complex epigenetic analysis is to be performed remaining that the results to be included in the presentation.

## P03.174

**Deletion of the CHRNA7 gene in a patient with Prader-Willi syndrome and epilepsy**

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Prader-Willi syndrome (PWS) is an imprinting disorder caused by loss of expression of paternally inherited contiguous genes from the chromosomal region 15q11-q13. In 70 percent of cases the affected patients carry an interstitial deletion on the paternal chromosome 15. Two recurrent deletions are commonly reported: one between the breakpoint 1 (BP1) to BP3 and the other between BP2 to BP3. We have recently shown that approximately 10 percent of the 15q11-q13 deletions are atypical and involve other breakpoints.

PWS and epilepsy is a rare occurrence reported in less of 10 percent of PWS. Recent studies have shown that CHRNA7 gene (cholinergic receptor neuronal nicotinic alpha polypeptide 7) which maps on chromosome 15q13.3 is involved in isolated epilepsy.

We report a case of a young girl affected with PWS and epilepsy. PWS was suspected because of neonatal hypotonia and craniofacial feature. The diagnostic was confirmed by methylation analysis and an unbalanced karyotype 45,XX,-15,der(10)t(10;15)(q26.3;q13) was found by cytogenetics analysis. We performed a Quantitative Multiplex PCR Short Fluorescent fragment methodology to analyse ten markers of the 15q11-q13 region including the CHRNA7 gene. We found a large atypical deletion leading to the loss of the CHRNA7 gene. Our results suggest that epilepsy in this case of PWS could be due to the haploinsufficiency of CHRNA7. At our knowledge, it is the first case of PWS and epilepsy with a demonstrated deletion of CHRNA7 gene. We propose to search for an atypical deletion in all patients with PWS and epilepsy.

## P03.175

**Deletion analysis using Quantitative Multiplex PCR of Short fluorescent Fragments (QMPSF) in patients with Prader-Willi syndrome: evidence for uncommon deletion types**

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Prader-Willi Syndrome (PWS) is a neurogenetic disorder that results from the absence of normally active paternally expressed genes from the 15q11-q13 chromosome region. About 70% of individuals have a paternally derived interstitial deletion of 15q11-q13. Two common classes of deletion has been reported: a type I between the breakpoint 1 (BP1) to BP3 and a type II deletion between BP2 to BP3. The deletion type status is commonly characterized by FISH on cultured cells and/or by microsatellites analysis on both patient and parental DNA. Because these two approaches don't allow the screening of the entire critical 15q11q13 region we believed that other types of deletion might be missed. We have recruited 66 PWS patients with routinely diagnosed 15q deletion and we have analyzed the critical region using a new molecular assay based on the QMPSF (Quantitative Multiplex PCR of Short fluorescent Fragments) method. This assay includes three multiplex PCR allowing amplification of 18 different gene-markers located from BP1 to telomeric to BP3 region. We found 16 PWS patients (24 %) with TI deletion, 44 (67 %) with TII deletion and 6 patients (9 %) carrying an uncommon 15q deletion. The ratio of TI/TII deletion was consistent with previous studies.

In conclusion, our QMPSF assay represents a powerfull tool for rapid detection and characterization for small rearrangements in 15q11-q13 region. It is particularly attractive to distinct different deletion types and useful for genotype-phenotype correlation studies in Prader-Willi syndrome.

## P03.176

**Evaluation of clinical findings in 17 Children with 22q11.2 deletion syndrome with/without congenital heart defects**

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Deletion of chromosome 22q11.2 (22q11.2 DS) is one of the most common microdeletion syndromes. It is estimated incidence of 1:4000-5000 live births. Approximately, 22q11.2 DS accounts for 5 % all newborns with congenital heart diseases (CHD) and 75 % of patients with 22q11.2 DS have CHD. The aim of this study was to evaluate clinical findings in individuals with 22q11.2 DS. We retrospectively evaluated both clinical findings and CHD in 17 children with 22q11.2 DS. 10 cases had CHD with/without conotruncal defect and 7 cases had without CHD. Major dysmorphic findings of the cases are hypertelorism, lateral displacement of the inner canthi, short palpebral fissures, swollen eyelids, dysmorphism of the nose, low set ears, minor ear-lobe anomalies and velopharyngeal insufficiency. We found that 12 cases had velopharyngeal insufficiency, 5 cases had hypocalcemia and one case with immune deficiency (Low T-cell level). Interestingly, we found that 3 cases (3/7) without CHD had dysmorphism of nose and all of the cases without CHD (7/7) had velopharyngeal insufficiency. But however only 5 case with CHD (5/10) had velopharyngeal insufficiency. In the study, we presented that dysmorphic features of 22q11.2 DS such as velopharyngeal insufficiency and dysmorphism of nose may be important sign for diagnosis of cases with 22q11.2 DS without CHD.

## P03.177

**DiGeorge syndrome presenting with laryngeal membrane**

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We present a case of DiGeorge syndrome with laryngeal membrane as additional element of the syndrome. The patient is the first child of healthy parents, male, born on term, spontaneously, BW 3730 gr, BL 51 cm, HC 37 cm, Apgar 9/10. Within first minutes of life baby cried without voice with occasional inspiratory stridor. Micrognathia, low set, small ears, hypertelorism, short palpebral fissures, blunted nose, short philtrum, high arched palate were also noticed. Endotracheal endoscopy showed omega epiglottis and laryngeal membrane occupying 50 % of laryngeal entrance. Cardiac ultrasound showed ventricular septal defect, atrial septal defect type II and right aortal arch. Fluorescence in situ hybridization (FISH) analysis revealed a deletion of chromosome 22q11.2.

Although rarely, laryngeal membrane, especially in combination with congenital heart defects should be considered for deletion 22q11.2.

## P03.178

**A case of Emanuel syndrome arising from 2:2 segregation of a paternal t(11;22)**

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Emanuel syndrome is an inherited chromosomal abnormality. It is the result of 3:1 meiotic segregation of a balanced translocation, t(11;22)(q23;q11). This rearrangement is the only recurrent non Robertsonian translocation in humans. Affected offspring usually carry a supernumerary derivative 22 chromosome and thus effectively trisomic for part of chromosome 22 and part of chromosome 11.

A 6 years female patient, presented with mental retardation, craniofacial abnormalities, with no cardiac abnormalities.

Cytogenetic analysis and FISH using WCP11, WCP 22, LSI N25 and LSI 22q13.3 revealed that the child had both translocation chromosomes, plus an additional copy of der(22). Her father had balanced translocation t(11;22), also her brother had the same balanced translocation. Her karyotype was 47,t(11;22)(q23;q11),+der(22). This rare karyotype can arise by 2:2 segregation in 1<sup>st</sup> meiotic division in the balanced translocation father, followed by nondisjunction at meiosis II in a balanced spermatocyte.

Genetic counseling and prenatal diagnosis and awareness of high risk breast cancer is very important in the families with balanced carrier of a t(11;22).

## P03.179

**Three cases of two unrelated families with a microduplication 22q11.2: developmental skull defects and phenotype variability**

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The 22q11.2 microduplication is a considerably new syndrome with an extremely variable phenotype ranging from normal to full clinical expression of MR/MCA syndrome. Commonly reported features are growth and motor delay, cognitive deficit, behaviour problems, hearing impairment, velopharyngeal insufficiency, urogenital anomalies. Here we report three cases of two unrelated families with 22q11.2 microduplication: a 10-year-old boy who inherited duplication from his healthy father and two brothers, 12-year-old and 2-year-10-months-old carrying the duplication of their healthy mother. Common features for all three were moderate psychomotor delay, widely open fontanel at birth with a delayed closure, and following dysmorphism: hypertelorism, strabismus, fifth finger clinodactyly, overlapping irregular toes. Additionally, the 10-year-old boy, presented unstable gait, asthma, pyloureteral reflux, ADHD, and both he and his father had open sagittal suture at birth. Extra features seen on the 12-year-old boy were lack of parietal and part of occipital bones, hypoplasia of temporal and occipital part of the brain, non-obstructive hydrocephaly, and hypospadias. His 2-year-10-months-old brother had prominent metopic suture, inverted nipples, deep palmar and plantar creases, two-phalangeal fifth finger, hypotonia, unstable gait, affect-respiratory episodes resembling epilepsy (without EEG changes), and dilatation of lateral brain ventricle. Duplication of respectively 3Mb and 2.5Mb of 22q11.2 chromosome region, "DiGeorge region", were detected by MLPA and BAC array (duplicated CHKAD-26-1 clone) and confirmed by FISH and MLPA. This report contributes with three new cases to the variable phenotype spectrum of microduplication 22q11.2 syndrome. To our knowledge it is the first report of developmental skull defects and microduplication 22q11.

#### P03.180

#### Prenatal diagnosis of a maternally derived unbalanced der(4)t(4;11) leading to an overlapping phenotype of Wolf-Hirschhorn and Russell-Silver syndromes in two siblings: novel recurrent translocation, aCGH and molecular cytogenetic analyses.

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Constitutional reciprocal translocations were thought to arise randomly with the only exception of recurrent rearrangements being the t(11;22). Recent evidence indicates that recurrent breakpoints may be more common than previously thought and mediated by non-B DNA structures, such as cruciforms, caused by palindromic AT-rich repeats. The clinical significance of these rearrangements may be underestimated because certain rearrangements, most notably subtelomeric translocations, can be difficult to detect by conventional G-banding.

We report on two siblings of same sex and apparently normal karyotypes both presented prenatally with severe intrauterine growth restriction (IUGR). The mother had an apparently balanced translocation, t(4;11)(p16.3;p15.5), and both sibs had an adjacent -1 segregation variant with loss of terminal 4p and gain of terminal 11p regions which was detected by aCGH and FISH using subtelomeric probes. Each of these rearrangements is associated with well known clinical phenotypes.

Paternal duplication of 11p15 is associated with the Beckwith-Wiedemann syndrome while maternal duplication is associated with Russell-Silver syndrome. As expected both fetuses presented with severe IUGR consistent with both Wolf-Hirschhorn and Russell-Silver phenotypes.

This familial case of what appears to be a novel recurrent translocation demonstrates the value of aCGH in the diagnosis of submicroscopic chromosome abnormalities in those patients for whom routine chromosome analysis does not adequately explain clinical findings. Breakpoint sequence analysis in our cases is compared to previously published cases and an analysis of the 4p and 11p regions for palindromic repeat sequences may help to elucidate mediators of this recurrent chromosomal aberration.

#### P03.181

#### Fourteen new cases contribute to the characterization of the 7q11.23 microduplication syndrome.

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Interstitial deletions of 7q11.23 cause Williams-Beuren syndrome, one of the best characterized microdeletion syndromes. The clinical phenotype associated with the reciprocal duplication however is not well defined, though speech delay is often mentioned. We present 14 new 7q11.23 cases with the reciprocal duplication of the Williams-Beuren syndrome critical region, 9 familial and 5 *de novo*. These were identified by either array-based MLPA or by array-CGH/oligonucleotide analysis in a series of patients with idiopathic mental retardation with an estimated population frequency of 1:13.000-1:20.000. Interchromosomal recombination is the preferred exchange mechanism by which the duplication arises. The *CYLN2* gene within the duplicated region is approximately 2 times overexpressed in carriers of the duplication. The expression of the *GTF2I* gene, just on the boundary of the duplicated region and thus with only 2 intact copies present in patients, was also significantly increased.

Variable speech delay is a constant finding in our patient group, confirming previous reports. Cognitive abilities range from normal to moderate mental retardation. The association with autism is present in five patients and in one father who also carries the duplication. There is an increased incidence of hypotonia and congenital anomalies: heart defects (PDA), diaphragmatic hernia, cryptorchidism and non-specific brain abnormalities on MRI. Specific dysmorphic features were noted in our patients, including a short philtrum, thin lips and straight eyebrows. In summary, patients with the reciprocal duplication of the Williams-Beuren region have a number of features in common, suggestive of a clinically recognizable syndrome

#### P03.182

#### Cardiovascular spectrum in Williams-Beuren Syndrome.

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The Williams-Beuren Syndrome (WBS) is a rare genomic disorder caused by a contiguous gene deletion on chromosome 7q11.23. The phenotype of WBS consists of typical dysmorphic features, supravalvular aortic stenosis, infantile hypercalcemia, a characteristic behaviour, and cognitive deficits relatively sparing auditory perception and cognition. We present four patients, two boys and two girls, ages ranged from 1 year to 14 years at the time of diagnosis. All patients were clinically diagnosed with WBS and confirmed by FISH testing with common elastin (ELN) gene probe. The cardiovascular spectrum of the patients varied and was the reason of genetic reward for 3 out of 4 patients. Supravalvular aortic stenosis was found in 1 case, with hypertrophic left heart and arterial systemic hypertension; 1 case had a severe form of pulmonary artery brachial stenosis and mitral valve prolapse, with surgical correction at the age of 10; 1 case had periferic pulmonary artery stenosis, renal artery hypoplasia and minor atrial septal defect which suggested the diagnosis in the first year of life and remained under supervision; 1 case had only a paroxysmic supraventricular tachycardia, and was diagnosed at the age of 14 due to cognitive deficits.

The cardiac findings from consensus criteria enabled a tentative clinical diagnosis of WS in 3 out of 4 patients and using FISH, the diagnosis was confirmed. The variable expression of the WBS cardiovascular spectrum requires periodic evaluation and impose a detailed analysis of the extra cardiac signs of these patients for a positive diagnosis.

## P03.183

**An atypical 7q11.23 deletion in a normal IQ Williams-Beuren syndrome patient**

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Williams Beuren syndrome (WBS, OMIM#194050) is a multisystemic neurodevelopmental disorder caused by a hemizygous deletion of 1.55 Mb on chromosome 7q11.23 spanning 28 genes. Haploinsufficiency of the *ELN* gene was shown to be responsible for supravalvular aortic stenosis and generalized arteriopathy, while *LIMK1*, *CYLN2* and *GTF2IRD1* genes were suggested to be linked to the specific cognitive profile and craniofacial features. These insights for genotype-phenotype correlations came from the molecular and clinical analysis of patients with atypical deletions and mice models.

We report the detailed clinical and cognitive examinations together with cytogenetics (FISH) and molecular (QPCR) analysis of a WBS patient showing mild WBS physical phenotype and normal IQ. He carries a shorter 1 Mb atypical deletion, which does not include the *GTF2IRD1* and *GTF2I* genes and only partially the *BAZ1B* gene. Our results are consistent with the previous hypothesis that hemizygosity of the *GTF2IRD1* and *GTF2I* genes might be involved in the facial dysmorphisms and in the specific motor and cognitive deficits observed in WBS patients.

## P03.184

**The elastin gene is not disrupted in a patient with a balanced translocation t(5;7)(q32;q11.23) and incomplete Williams-Beuren phenotype**

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The Williams-Beuren syndrome is a complex developmental disorder with multisystemic manifestations including supravalvular aortic stenosis (SVAS), a typical face and a specific cognitive phenotype. Elastin is one of the deleted genes in this contiguous syndrome whose haploinsufficiency causes cardiovascular malformations. Correlations between deleted genes and observed phenotypes are not well established. Here, we report the case of a 5 years and 6 months old boy referred to us for psychomotor delay and mental retardation.

Physical examination showed a dysmorphic face with a broad forehead, full cheeks, epicanthus, depressed nasal bridge, open mouth, protruding lower lip. He also has a severe myopia. A mild mental retardation was noticed, as our patient is attending school but having attention difficulties.

Neither cardiovascular malformation nor abdominorenal anomalies were found by ultrasound examination. However, karyotype showed a reciprocal translocation between chromosomes 5 and 7: 46,XY,t(5;7)(q32;q11.23). After FISH experiments with Vysis *ELN* probe and Vysis *EGR1* probe, we concluded that the breakpoint on chromosome 7 was proximal to *ELN* and on chromosome 5 distal to *EGR1*. Further FISH studies showed that the breakpoint on chromosome 7 is proximal to the BAC RP11-313P13 (i.e 1Mb from *ELN*). We are studying the exact breakpoint to determine the disrupted region which could help us to better correlate features in Williams syndrome with candidate genes.

## P03.185

**Azoospermia due to a de novo balanced reciprocal translocation (Y;3)(q12;q22)**

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It has been suggested that among infertile couples 4.5% of males and 6% of women have some sort of chromosomal aberrations. Aside from sex chromosome abnormalities (males, 2% and females, 4.5%), balanced reciprocal translocations are the most frequent chromosomal aberrations with the frequency of about 1% for males and 0.7 for fe-

males. Although all of the chromosomes can be involved in reciprocal translocations, chromosomes 12, 22 and Y are involved more often than expected on the basis of their relative lengths. A balanced reciprocal translocation between chromosome Y and autosomes has been demonstrated. The association between translocation (Y;1) and azoospermia also has been suggested.

We report a balanced reciprocal (Y;3) translocation associated with azoospermia which we could not find similar published case in the literature. A 30-year-old man was referred to our department due to infertility. Physical examination of patient was unremarkable. Testicular sonography revealed a homogenous echo, and mild bilateral varicoceles. The size of the right testis was 47x25 mm, and the left was 47x28 mm. His semen analysis showed: volume ( 0.8 ml ), count ( 0.05 mill/ml ), motility ( 100% non-motile ). The levels of his follicle-stimulating hormone, luteinizing hormone, prolactin and testosterone were within normal limits. Chromosomal study on his peripheral blood was performed, using standard G-banding techniques, and revealed that all his cells have a balanced translocation between chromosomes number 3 and Y with a 46,X t(Y;3)(q12;q22) karyotype.

## P03.186

**AZF microdeletions in Iranian non-obstructive azoospermia patients**

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Genetic factors cause about 10% of male infertility. In the present study the existence and or absence of 8 sequence tagged sites markers representing AZF regions (AZFa, AZFb, AZFc and AZFd) was studied using Multiplex PCR. Of the total 106 azoospermic men analyzed, 14 individuals (13.21%) showed Y chromosome deletion, of which deletion in AZFb region was the most common (71.43%) followed by AZFc (50%) , AZFd (42.86%) and AZFa (21.43%). None of these microdeletions was detected in the control fertile group. Also in the case of AZFc partial deletions, no significant statistical correlation was observed between the incidences of gr/gr, b1/b3 and b2/b3 deletions and male infertility (P values were 0.153, 0.465, and 0.447 respectively).

## P03.187

**Molecular and cytogenetic analysis of Y chromosome abnormalities in males with reproductive disorders**

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**Introduction:** Approximately 10-15% of couples are affected by infertility. The male factor of infertility (the azoospermia factor, AZF) was mapped to three different subregions (AZFa, AZFb, AZFc) in Yq11 which are associated with spermatogenic failure. The aim of this study was: 1) to determine the prevalence and type of microdeletions of the Y chromosome of Czech males with reproductive disorders; 2) to describe cytogenetic abnormalities.

**Methods:** Karyotypes and AZF microdeletions were analyzed in 136 males from patients with azoospermia and oligospermia. Genomic DNA was extracted from peripheral blood samples and two multiplex polymerase chain reactions (PCR) were performed using sequence tagged sites (STSs) primers to confirm the presence or absence of Y chromosome microdeletions (Simoni et al., 1999).

**Results:** Microdeletions of Y chromosome were detected in ten (7.35%) patients. Among these, one had deletions in AZFa, three in AZFc, three in AZFb+c and two in AZFa+b+c regions. In one case, the partial deletion in AZFb (sY134) region was found. We present a molecular and cytogenetic analysis of a mosaic 45,X/46,X,idic(Y) in patient with isodicentric Y chromosome with two short arms and small portion of the long arm (microdeletions in Yq11).

**Conclusion:** Screening for microdeletions in AZFa, b and c region of Y chromosome showed a big variation among different studies. Our study did not find any population dependent differences in the preva-

lence and type of AZF microdeletions comparing to present world studies and the incidence of the microdeletions in Czech infertile males falls within the range published in other countries.

#### P03.188

#### Male Infertility - an association study by GGC and CAG repeats from gene AR analysis

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**Background:** The human androgen receptor (AR) gene is located on X chromosome, region q11-12 and contains two polymorphic trinucleotide repeats of CAG and GGC, which code for polyglutamine and polyglycine tracts in the N-terminal domain where the receptor activity resides. Longer repeats induce decrease of transactivation function in the AR receptor and weaken an anti-proliferative effect on various steroid-related tissues. Shorter GGC and CAG repeats result in higher receptor-activity of AR that inhibits the growth of these steroid-related tissues. In fertile men the number of CAG repeats varies between 10 and 35 while GGC repeats varies between 4 and 24.

**Methods:** A group of 108 infertile men and other composed of 99 subjects with proven fertility were selected to be used in this study. Differences in CAG and GGC frequencies were calculated with Fisher's exact test. P<0.05 was considered statistically significant.

**Results:** No significant difference was found between patients and controls in distribution of CAG and GGC, when analysed separately. However, the analysis of the joint distribution of CAG and GGC (table 1) showed that the distribution of particular haplotypes is significantly different between patients and controls.

**Conclusion:** Small sizes of both CAG and GGC repeats seems to be able to predispose to the male infertility while the bigger sizes, mainly for CAG repeats, will be able to have a protector effect in male infertility.

#### P03.189

#### A case of azoospermia associated to Yp duplication and partial Yq deletion

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Structural abnormalities in Y chromosome are involved in male infertility.

The most frequently reported anomalies concern the Y long arm with deletions especially in the AZF region. Anomalies in the short arm are rather rare.

We report a case of infertility in a 30 years old man. Spermogram in this patient showed azoospermia.

Physical examination found small testis confirmed by ultrasound examination, normal stature and normal mental development. He was operated at age of 15 years old for cryptorchidism.

Karyotype performed on peripheral blood lymphocytes was interpreted as: 46,X,der(Y).

FISH was done for further characterization using SRY, Yp telomeric and Yq telomeric probes.

We found on the rearranged Y chromosome 2 SRY signals, 2 Yptel Signals and no Yqtel signal.

We concluded that the rearranged chromosome contains a duplication spanning at least the region from the Yptel to the SRY locus which contains approximately 2,7Mb.

Our clinical data with these findings led us to study the AZF region. Using 9 STS markers, we found a partial deletion. In fact, no deletion was found in AZFa. However, in AZFb one STS marker (sY135) is present and two others (sY127 and sY142) are deleted. Markers (sY277, sY158) tested in AZFc region are deleted.

Thus our patient harbour a duplication of the Yp region (Yptel>Yp11.31) and a deletion in the Yq region (Yq11.222>Yqtel) associated to azoospermia.

Few cases of i(Yp) have been reported but to our knowledge, this is the first case with duplication of Yp and Yq deletion.

#### P03.190

#### Pericentric inversion in chromosome 1 in a patient with azoospermia

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A 36 year old patient was referred to our clinic for genetic counseling due to infertility. Patient reported no history of inflammation or damage of the testis. Sperm analysis established azoospermia. Cytogenetic analysis using GTG banding showed 46, XY karyotype with a pericentric inversion of chromosome 1 - inv(1)p13q23. Both parents of the proband had a normal karyotypes.

Pericentric inversions of human chromosomes was frequently reported in literature, however clinical significance is yet to be established due to apparent lack of phenotypic manifestations. Chromosome 1 was rarely associated with phenotypic alterations and here we present an interesting case of infertility associated with this abnormality.

#### P03.191

#### Estimation of genetic abnormalities in male infertility

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Twenty oligozoospermic patients were referred to us in order to evaluate some of the commonest genetic abnormalities that lie behind many cases of male infertility. Microdeletions of the Y chromosome and karyotypic abnormalities are the most frequent genetic causes of defective spermatogenesis. Men with bilateral congenital absence of the vas deferens are probable CF carriers and patients should be tested for CFTR gene mutations. Additionally, sperm DNA fragmentation and elevated aneuploidy rates are being increasingly recognized as important causes of male infertility.

Our cohort comprised of 10 patients with severe and 10 patients with moderate oligozoospermia. No patient had deletions for the 5 screened regions of Y chromosome (AZFa, AZFb, AZFc, SRY, Yq12). None of the commonest mutations of CFTR gene was detected. All were karyotypically normal. DNA fragmentation measured with TUNEL assay was not significantly elevated compared to 20 samples with normal sperm parameters and ranged from 2%-18.8% (cut-off limit: 20%), with the exception of one patient with 40.4%. The rate of aneuploidy was measured using FISH on chromosomes 13, 15, 16, 18, 21, 22, X and Y. Six patients (30%) had a significantly increased aneuploidy rate compared to normal controls: 4 patients were aneuploid for chromosomes X and Y, 1 patient for chromosomes 22, X and Y and 1 patient for chromosomes 15, 18, X and Y.

These preliminary results prove the diagnostic importance of genetic studies in investigating male infertility and suggest that molecular cytogenetics (FISH) should be incorporated in sperm analysis in routine basis.

#### P03.192

#### The Relationship between Sperm mtDNA Mutations, Sperm Parameters and Genetic Testing Results in Male Infertility

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Infertility a significant problem, affecting up to 15% of couples of reproductive age. Common causes for male infertility are genetic factors (chromosomal abnormalities, Y chromosome microdeletions), hormone and/or receptor problems, sperm production, delivery and motility problems. Sperm mitochondria plays a significant role for sperm motility as oxidative fosforilation (OXPHOS), driven by mitochondria, provides energy for sperm. Recently, the role of mitochondrial DNA defects related OXPHOS enzymatic complexes in male infertility are in great interest. In this study , we aimed to reveal relations between DNA abnormalities, sperm parameters and genetic testing results.

30 patients with both structural and numerical anomalies (19 normo-

spermia, 11 oligospermia) and 30 controls were recruited. Chromosome analysis were performed from peripheral blood and Y- chromosome deletions were detected by using commercial Kit. Sperm DNA purifications were carried out from both patients and controls. Mitochondrial ND1, ATPase6 and Cytb genes were amplified and the amplicons were analysed by direct sequencing.

All the participants were 46, XY with no Y- deletions. Some common polymorphisms/ mutations including patients and controls were detected in ND1, ATPase6 and Cytb genes.

No correlation was found between sperm parameters and mtDNA mutations in this study. It is suggested that low sample number may be a reason. We are looking forward to increase the number of cases and determine D-loop which is another important locus of mtDNA in terms of male infertility.

#### P03.193

#### CAG repeat length in the androgen receptor gene in infertile men in Serbia

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The purpose of this study was to evaluate CAG repeat length in the androgen receptor gene in Serbian men with severe oligozoospermia. Initially, 180 infertile patients were included in the study and spermogram has been performed in order to determine the sperm density. Patients were excluded if clinical evidence of obstructive azoospermia, known cytogenetic defects, Y chromosome microdeletion or abnormal hormonal parameters were present. Control group of 100 age-matched men who had fathered at least two children was also analyzed for CAG repeat length in the androgen receptor gene. The screening was performed in 106 selected patients with idiopathic infertility by polymerase chain reaction (PCR) method on DNA extracted from peripheral blood. The mean CAG repeat length in the androgen receptor gene in infertile group ( $20.2 \pm 1.2$ ) did not differ significantly than in control group ( $21.0 \pm 0.7$ ) ( $t=0, 0978, p>0.05$ ) Conclusion: No significant correlation was found in CAG repeat length between infertile men and controls in Serbian population.

#### P03.194

#### Sperm mitochondrial DNA mutations associated with reactive oxygen species (ROS) and sperm DNA damage in male infertility

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Excess Reactive oxygen species (ROS) in the semen is believed to affect the sperm function. However, the mechanism behind the elevated ROS and impaired sperm parameters is not clear. The present study was aimed to find the correlation between ROS levels, mtDNA mutations and sperm DNA damage in semen of idiopathic infertile men. Study included 50 idiopathic infertile men and 45 fertile controls. ROS was measured by chemiluminescence assay. Whole sperm mtDNA was sequenced by standard PCR-DNA sequencing method. Sperm DNA damage was studied by COMET assay. Infertile group showed significant difference in the sperm parameters compared to control men. Infertile group showed significantly ( $p<0.001$ ) higher ROS levels ( $157.76 \pm 78.88$  cpm) /  $10^6$  spermatozoa compared to fertile controls ( $4.98 \pm 1.82$  cpm) /  $10^6$  spermatozoa. mtDNA sequencing revealed that 66% of the infertile group harboured one or more nucleotide changes in the mitochondrial genome compared to control men inspite some common nucleotide changes (A750G, A4769G) in both the groups. An average of 60% sperms showed (C+D) grade-higher DNA damage comet than the control group, which had an average of 15% sperm cells showing C+D grade comet. Higher ROS in the semen infertile men compared to the controls may be due to large number of nucleotide changes in the mtDNA. As sperm DNA integrity is important for successful fertilization, screening mtDNA mutations in infertile men with severe oxidative stress may help in the better management for treatment/ART

#### P03.195

#### MTHFR 5'UTR hypermethylation in testicular biopsy of Iranian patients with nonobstructive azoospermia: the role of epigenetics in male infertility

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Genetic factors involved in male infertility are not yet completely explored. MTHFR has been shown to be involved in male infertility through the pathway of folate metabolism. However, as contradictory results are reported regarding polymorphisms of this gene, other mechanism of pathogenesis like as promoter hypermethylation can be involved. In this study we explored methylatin status of 5' UTR region of MTHFR in male patients with nonobstructive azoospermia with patient who had no known anomaly of spermatogenesis.

#### Materials and method:

DNA from peripheral blood of 50 patients and 50 controls where extracted by salting out method. DNA from testicular biopsy samples of 35 patients with nonobstructive azoospermia and 5 patients with obstructive azoospermia and normal spermatogenesis were extracted using Roche DNA extraction kit. MSP was performed using primers which had been designed to hybridize to CpG island in 5'UTR of MTHFR. PCR products were electrophoresed on 8% bisacrylamide.

#### Results:

Blood samples in patient and control groups showed no difference in methylation. 53% of patients with nonobstructive azoospermia showed the presence of methylated allele compared to 0% in control group. This result confirms the presence of hypermethylation in testicular sample of patients with azoospermic male infertility ( $P\text{-values}=0.03 < 0.05$ ).

**Discussion:** This is the first report to show the implication of epigenetic silencing of MTHFR in patients with azoospermic male infertility. This results could be considered as a step toward a tailored therapy in patients with azoospermia.

#### P03.196

#### Are Y chromosomal fragments involved in Müllerian aplasia?

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Müllerian aplasia (MA) is a congenital abnormality of the female genital tract profoundly affecting a woman's life. Due to reasons still unknown, the Müllerian (paramesonephric) ducts regress during fetal development resulting in absence of upper two-thirds of the vagina and functional uterus. Without treatment intercourse is difficult and pregnancy is only possible through surrogacy. The incidence of MA is 1:5000 newborn girls and the syndrome is often associated with renal and skeletal malformations.

Previously the Y chromosomal *TSPY1* gene has been reported to be involved in the development of MA. The aim of this study was to investigate the presence of possible Y chromosomal fragments in 110 Finnish MA patients. In addition to attempting to amplify fragments of the previously reported *TSPY1* gene, we also included 38 additional loci in order to cover the entire Y chromosome. This investigated sample set comprises, to our knowledge, a significantly larger sample series than in any individual studies published to date.

None of our patients showed presence of the *TSPY1* gene fragment or any of the 38 additional Y chromosomal markers, suggesting that Y chromosomal genetic factors are not associated with development of MA in these patients. However, we can not completely exclude the role of Y chromosome fragments in the disorder.

#### P03.197

#### Oxidative stress and apoptosis in spermatozoa of infertile men.

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**BACKGROUND:** DNA damage in sperm originates due to improper maturation, oxidative stress and apoptosis. DNA damage has recently been proposed as a better diagnostic and prognostic marker of sperm

quality as compared to conventional semen analysis. The aim of this study was to examine the role of apoptosis and reactive oxygen species (ROS) in inducing DNA damage in ejaculated spermatozoa. METHODS: We examined spermatozoa for apoptosis by flow cytometry, DNA damage by comet assay and raw semen for Reactive Oxygen Species (ROS) by chemiluminescence in 43 idiopathic infertile patients having normal semen parameters, 67 infertile men with abnormal semen parameters and in 29 fertile controls. RESULTS: Apoptosis in infertile men with normal semen parameters was significantly higher ( $p=0.009$ ) as compared to controls but not significantly different from infertile men with abnormal sperm parameters ( $p=0.317$ ). ROS values were significantly correlated with apoptosis levels in patients with normal and abnormal semen parameters. ( $p=0.023$ ;  $p=0.0121$  respectively) but not in the fertile controls ( $p=0.067$ ). Non significant difference in Olive Tail Moment (OTM) was obtained in patients with normal and abnormal semen parameters. However significant difference for OTM was observed in the fertile controls and patients with normal and abnormal semen parameters ( $p=0.014$ ;  $p=0.029$ ). CONCLUSION: DNA damage analysis provides additional dimension to semen analysis and has the advantage of rapidly performed and interpreted. Thus DNA damage evaluation should be included in the diagnostic workup of infertile men.

#### P03.198

#### Analysis of 5T allele of the *CFTR* gene intron 8 in men with azoospermia and oligozoospermia from Ukraine

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Men with azoospermia or oligozoospermia due to germ cell failure were suggested to have an increased risk of carrying *CFTR* gene mutations. 5T is one of the alleles found at the polymorphic Tn locus in intron 8 of the *CFTR* gene. A stretch of 5, 7 or 9 thymidine residues is found at this locus. Less efficient splicing will occur when a lower number of thymidines are found, resulting in *CFTR* transcripts that lack exon 9 sequences.

In the present study we evaluated the incidence of the 5T allele in men with azoospermia and oligozoospermia from Ukraine.

Isolated DNA from blood samples of 153 infertile men (60 - azoospermia and 93 - oligozoospermia) and of 102 fertile men (control group) has been amplified by polymerase chain reaction targeting the 5T allele of the *CFTR* gene intron 8. For the 5T allele discrimination fragment analysis of Cy5-labeled PCR products on an automated DNA analyzer "A.L.F.-express" were used.

The percentage of patients with infertility who had 5T allele was significantly higher ( $p<0.05$ ) than that in the control group (13.1% vs. 6.9%). The proportion of patients with azoospermia (15.0%) and oligozoospermia (11.8%) who had the 5T allele was higher comparing to the control group (6.9%), but not significantly different ( $p>0.05$ ). Obtained data suggests that 5T allele could be involved in the process of spermatogenesis or sperm maturation in azoospermic and oligozoospermic males.

#### P03.199

#### The frequency of b2/b4, b2/b3 and gr/gr deletions in Russian infertile men

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Introduction: Y chromosome microdeletions are commonest genetic cause of male infertility. In contrast to complete AZF deletions partial AZFc region deletions is still under evaluated. The aim of our study was to analyze of frequency of partial AZFc deletions in Russian infertile men.

Materials and Methods: We investigated a cohort of 1510 Russian men from infertile couples. Complete AZF deletions were detected according to Laboratory guidelines for molecular diagnosis of Y-chromosomal microdeletions (EAA/EMQN, 1999), with some modifications. Partial deletions in AZFc region were tested by multiplex PCR of following STS loci: sY142, sY1197, sY1192, sY1291, sY1206, sY1054 and sY1125.

Results: In total AZF deletions were detected in 268 (17.8%) examined individuals. Complete AZFc (b2/b4) deletions were found in 54 (3.6%)

infertile men. Other complete (classic) AZF deletions (AZFa, AZFb and AZFb+c) were revealed in 19 (1.3%) patients. Partial AZFc deletions were detected in 195 (12.9%) in examined individuals. Most common types of partial AZFc deletions were b2/b3 and gr/gr deletions. Their frequencies were 8.5% and 3.6%, respectively. Deletion b1/b3 was revealed in one patient. Other types of deletions partially covering AZFc region were detected in 11 (0.7%) examined men. Various degrees of spermatogenesis defects (from asthenozoospermia to azoospermia) have been found in patients with partial AZFc deletions.

Conclusion: Obtained data demonstrated high prevalence of partial AZFc deletions in Russian infertile men. Partial deletion b2/b3 is commonest the Y chromosome microdeletion among Russian infertile men.

#### P03.200

#### A Case of 46,XX Male Syndrome

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46,XX male syndrome is a rare sex chromosome disorder occurring about 1 in 25 000 males. XX male syndrome mostly results from unequal crossing over between X and Y chromosome during male meiosis. Point mutations, deletions or translocations of sex determining region Y gene (SRY) the most common causes of sex reversal. Approximately 80% of 46,XX males have translocation of SRY region onto an X chromosome. SRY positive chromosomal aberrations arise due to unequal recombination between Xp and Yp terminal regions during paternal meiosis. In this report, we present the clinical, cytogenetical, molecular cytogenetical, molecular data and X chromosome inactivation pattern of a 16-year-old patient referred to urological clinic for evaluation of small testes and small penis. Chromosomal analysis revealed 46,XX karyotype. SRY amplification was positive and is confirmed by fluorescence in situ hybridization (FISH). FISH test showed the presence of SRY region translocated to the short arm of the X chromosome. Analysis of these cases illustrated that conventional cytogenetic, FISH and SRY amplification techniques are useful for accurate diagnosis and genetic counseling.

#### P03.201

#### Ambiguous Genitalia: Diagnosis and management with special reference to parental consanguinity and age at diagnosis

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Diagnosis of ambiguous genitalia (AG) represents an enormous challenge. One hundred six cases were ascertained over the past 10 years (1999-2008) in three Arab countries (Egypt, Libya, and Saudi Arabia) by clinical evaluation, blood karyotyping, hormones and biochemistry, pelvic imaging, and gonadal biopsy and SRY detection when indicated. Results revealed MSHP in 66 cases, FSHP in 29 cases, 5 cases with genetic syndromes, 5 cases with gonadal dysgenesis (GD) and one true hermaphrodite (THF) with the 46,XX karyotype. Age of diagnosis ranged between newborn and 36 years (mean is 5.6 years). Parental consanguinity was observed in 82 cases (74 of them showed autosomal recessive disorders as a cause for their genital ambiguity). Six families had more than one sib affected (2-6). Proper gender reassignment was performed in 15 cases where patients with complete form of TFS were assigned the female sex while partial TFS, GD and THF were assigned either gender according to the feasibility of surgical reconstruction, the functioning gonads, pattern of expected hormonal pubertal changes and the patient and family desire (gender identity). Four cases rejected gender reassignment due to late diagnosis (12-23 years), religious and psychosocial reasons. This study highlights the high frequency of AG due to parental consanguinity and the late onset at diagnosis in Arab countries which might correlate with religious

and social factors. It also emphasizes the importance of considering gender role and gender identity during sex reassignment especially in late diagnosis to avoid the deleterious psychological impact that might reach suicidal attempts.

#### P03.202

##### A rare deletion in X chromosome in a child with sexual reversal

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A baby was referred to our laboratory because of ambiguous genitalia. There was no relevant family history.

Material & Methods: lymphocyte cultures from the patients were set up in RPMI1640 supplemented with 20% FBS. High resolution chromosome banding was performed.

Result: The karyotype was determined as: 46,X,del(X)(q26). Sonography revealed that the baby had a normal uterus and ovaries, but no testes were observed.

Discussion: The deletion of this region has not previously been reported as a cause for the ambiguous genitalia.

## P04. Reproductive genetics

#### P04.01

##### Multiple aneuploidy in spontaneous abortions is associated with advanced maternal age: data retrieved from analysis of 600 consecutive cases

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Aneuploidy and polyploidy together represent the most common genetic cause of spontaneous abortions (SA). However, the origin of aneuploidy in human conceptions remains incompletely understood. We have hypothesized the existence of either meiotic or mitotic instabilities produced by environmental factors, or maternal age, may be associated with germline aneuploidy or post-zygotic aneuploidization (polyploidization) of fetal tissues. Maternal age has the potential to be more evident in cases of multiple aneuploidy (MA) or polyploidy (MP). To test the hypothesis, we have investigated the karyotype in 600 samples of SA by interphase FISH taking into account maternal exposure to common environmental mutagens and maternal age. Aneuploidy and polyploidy were detected in 50.1% of cases. Both MA and MP were found in 9.4% of cases of SA. More precisely, MP was found in 3.5% of cases, autosomal and autosomal/gonosomal MA - in 3.2% of cases, and pure gonosomal MA - in 2.7% of cases. A sociogenetic analysis has not revealed correlations between incidence of MA/MP and maternal tobacco exposure, alcohol and irradiation. Analysis of maternal age in three aforementioned groups of MA/MP has shown that pure gonosomal MA and MP do not exhibit higher prevalence among mothers with advanced age. On the other hand, autosomal and autosomal/gonosomal MA has demonstrated a 2-fold-increased incidence in mothers with advanced age ( $P<0.05$ ). The data suggest advanced maternal age increases autosomal aneuploidy risk and suggests autosomal MA could serve as a model for uncovering the origin of aneuploidy.

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#### P04.02

##### Investigation of 21-hydroxylase gene mutations in assisted reproductive techniques.

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The incidence of classic and nonclassic forms of congenital adrenal hyperplasia (CAH), which are caused mostly by mutations of the 21-hydroxylase gene (CYP21A2), is very high, and in some populations much higher than the frequency of cystic fibrosis. In spite of this fact, CAH is not included in genetic investigations of oocyte donors in as-

sisted reproductive techniques (ART). Moreover mutations of CYP21 can lead to various reproductive disorders in females so they may be found in ART patients.

We tested 9 frequent mutations of CYP21 gene using PCR and RFLPs in 8 unrelated females with different extents of 21-hydroxylase deficiency. We also performed a retrospective investigation of 43 oocyte donors. We determined that one female with classic virilizing form of CAH was a compound heterozygote - P30L/IVS2AS,A/C-G,-13 linked with 8bp del. Another female with a nonclassic form of CAH had a P30L/V281L genotype. Linked mutations IVS2AS,A/C-G,-13 and 8bp del were revealed in a patient with sexual maturation delay. We did not find any mutations in 4 females with hyperandrogenism. Four oocyte donors (9.3%) were carriers of CYP21 mutations: one with the severe mutation R356W and 3 donors with the mild mutation V281L as a heterozygote.

Genetic investigation of females with CAH and suspicion of CAH revealed CYP21 mutations, including severe mutations. Our results indicate that it seems to be necessary to investigate CYP21 mutations in oocyte donors.

#### P04.03

##### DNA damage in men exposed to occupational conditions of toxic fumes.

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**BACKGROUND:** Occupational exposure to mutagenic/carcinogenic fumes which include polycyclic aromatic compounds that bind to DNA and forms chemical-DNA adducts leading to DNA breaks are a safety concern for car mechanics and petrol pump workers. **AIM:** To investigate the association between exposure to vehicle fumes with sperm DNA and lymphocyte DNA damage in car mechanics and outdoor workers at the petrol pump. The sperm parameters of the exposed group were also compared to healthy controls. **METHOD:** Sperm and lymphocyte DNA damage was analyzed by comet assay in 23 car mechanics and petrol pump workers having an exposure period of minimum 1 year and 17 unexposed healthy controls. Semen analysis was done according to WHO 1999 criterion. **RESULTS:** The exposed group of men have significantly higher lymphocyte and sperm DNA damage as compared to unexposed controls ( $p=0.0199$ ,  $p=0.0278$  respectively). The exposed group has higher number of sperm parameter pathologies. The motility and normal morphology was significantly lower in workers and mechanics as compared to controls ( $p=0.021$ ,  $p=0.039$ ). Non significant association between sperm concentration was observed in the exposed and unexposed groups ( $p=0.065$ ). **CONCLUSION:** The assessment of DNA damage by comet assay in the studied occupationally exposed group provides an important tool for precise assessment of the associated potential health risks.

#### P04.04

##### Single nucleotide polymorphisms in promoters of MMP-2 and MMP-9 are associated with endometriosis risk

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**Background:** Matrix metalloproteinases (MMPs) are proteolytic enzymes that may contribute to the development of endometriotic lesions. The aim of this study was to investigate the associations between the promoter region polymorphisms of MMP-2 and MMP-9 and risk of endometriosis.

**Methods:** 150 Estonian patients with endometriosis and 199 healthy women were studied. PCR based restriction fragment length polymorphism analysis was used to detect SNPs in promoter regions of MMP-2 (rs243866, rs243864, rs2285053) and MMP-9 (rs3918242). All statistical tests were carried out using SPSS 17.0 program (SPSS Inc., Chicago, IL, USA).

**Results:** Women with MMP-2 -735 (rs2285053) TC and TT genotype had a lower risk ( $p=0.016$ ) of developing endometriosis compared to the CC genotype carriers. The distribution of MMP-9 -1562 (rs3918242)

genotypes and allele frequencies was similar in both groups. However, after dividing patients into subgroups according to the severity of disease (minimal-mild and moderate-severe) the multinomial logistic regression analysis showed women with TT genotype or TT/TC genotype had higher risk to develop moderate-severe endometriosis than CC genotype carriers ( $p=0.013$  and  $p=0.027$ , respectively). There were no significant differences in genotype and allele frequency distributions of the *MMP-2* -790 (rs243864) and -1575 (rs243866) polymorphisms between studied groups.

**Conclusions:** Current study showed that the presence of T-allele in *MMP-2* -735 polymorphism was associated with decreased probability to endometriosis, while the T-allele in *MMP-9* -1562 polymorphism was related to elevated risk of severe form of endometriosis.

#### P04.05

#### Follicle -stimulating hormone receptor gene polymorphism and Ovarian response to controlled ovarian hyperstimulation for IVF

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The aim of study was to investigate the association between FSH receptor (FSHR) gene polymorphism at position 680 and the outcomes of controlled ovarian hyperstimulation (COH) for in vitro fertilization and embryo transfer (IVF-ET) in Iranian women.

**Materials and methods:** one hundred and eight patients under 35 years old who underwent IVF-ET procedures were included in this study. The hormonal profile and treatment of all patients were analyzed and FSHR polymorphism was examined by PCR-RFLP. Women from all groups were classified as Asn/Asn, Asn/Ser, and Ser/Ser genotype.

**Result:** Our study showed that all of patients in Asn/Asn group were normal responder and in Asn/Ser group 64.8% were normal responder and 21.1% and 14.1% were poor and hyper responder respectively.

In Ser/Ser group we didn't have normal responder and 46.7% of these patients were poor responder and 53.3% were hyper responder.

**In Conclusion:** FSH receptor polymorphism is correlated with response to ovarian stimulation by FSH.

#### P04.06

#### Intracytoplasmic Sperm Injection: What Are The Risks? A Retrospective Tunisian Study

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**Background:** Over a million children have been born from assisted reproductive technology (ART) worldwide. Children conceived through ART comprise as many as 1% to 2% of total births in some countries. Newer techniques being introduced appear less 'natural', such as intracytoplasmic sperm injection (ICSI), but there is little information on these children beyond the neonatal period in Tunisian population.

**Objectives:** We aimed to perform a detailed assessment of children born from ICSI. One of the primary objectives of the study was to assess whether ICSI is associated with significant health problems.

**Material and methods:** Here we report findings surrounding growth, morbidity, physical defects and karyotype in the 69 ICSI conceived children.

**Results:** The children conceived by ICSI examined had experienced an excess of malformations in the (boys') urogenital system. In addition, all karyotypes were normal except for one detected mosaicism. A detailed physical examination revealed no further substantial differences between the groups.

**Conclusion:** The results of this study on outcome of ICSI pregnancies are in line with earlier reports, except that no sex chromosome abnormalities were found. Assessment of singleton ICSI children was generally reassuring, however, we found that ICSI children presented with more congenital malformations and were more likely to need health care resources than naturally conceived children.

#### P04.07

#### Superovulation in mice alters the methylation pattern of imprinted genes in the sperm of the offsprings

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Imprinting is form of gene regulation that mediates a parent-of-origin-dependent expression of the alleles of specific genes. The assisted reproduction techniques have the potential to interfere with imprinting reprogramming. In the present study, the possible deleterious effects on imprinting of superovulation were evaluated in the mouse. Superovulation was followed by natural mating and in vivo development. Then, possible methylation defects in the differentially methylated domains (DMDs) of 2 paternally (H19 and Gtl2) and 3 maternally (Peg1, Peg3 and Snrpn) imprinted genes were tested in the male offsprings. The CpGs methylation status within the 5 gene DMDs was analyzed in the liver, skeletal muscle, tail and sperm DNAs by pyro- or bisulfite sequencing. In the liver, skeletal muscle and tail of controls, the percentages of methylated CpGs were close to the theoretical expected value of 50% and no effect of superovulation could be observed. In the sperm of controls the percentages of methylated CpGs were close to the theoretical values of 100% and 0% in paternally or maternally imprinted genes, respectively. Superovulation did not induce any change in the numbers of methylated CpGs of Gtl2 and Peg3 genes but, interestingly, induced a significant 6 % decrease in the number of methylated CpGs of H19 and significant 2.5- and 5.3-fold increases in those of Peg1 and Snrpn, respectively. A better understanding of the mechanism by which possible superovulation-induced imprinting defects in the oocytes are transmitted to the male germline of the following generation would certainly help to better understand imprinting defect transmission.

#### P04.08

#### Preventive genetic screening of a human male carriers of balanced structural aberrations

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Carriers of balanced structural aberrations are often infertile and have a higher risk of birth of a child with an unbalanced chromosomal aberration. Cytogenetic screening of all infertile couples is standard routine now. Karyotype is determined in 650 infertile couples per year, chromosomal abnormalities are detected in 10 % of these patients. Structural aberrations are more frequent in men, whereas numeric aberrations prevail in women. Approximately 10 infertile males - carriers of balanced structural aberration are gathered a year in our department. Concerning the preventive care, an examination of karyotype is recommended within prenatal screening in these couples, and the preimplantation genetic diagnosis in case of IVF cycles.

The individual genetic risk can be assessed by determination of frequency of chromosomally unbalanced sperm in male carriers of balanced aberrations. Sperm samples of 40 male carriers of balanced translocations were examined using FISH, the frequencies of spermatozoa with pathological chromosomal content ranged from 4.2 to 70.3 %. The sperm FISH analysis of meiotic segregation and aneuploidy helps to personalize the reproductive risk and choose the most effective assisted reproduction strategy, for example the use of preimplantation genetic diagnosis or a donor of sperm.

#### P04.09

#### Karyotype abnormalities in reproductive failure

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This study analyses the prevalence of karyotype changes among patients referred for reproductive failure. Each patient was screened for karyotype changes by GTG banding on cultured lymphocytes from peripheral blood. No subjects presented in this report had obvious phenotypic signs of constitutional chromosomal abnormalities.

**Results:** 25 of 680 couples (3.67%) had one partner carrying a chro-

mosomal change. The frequency of chromosomal abnormalities in men and women were similar.

chromosomal rearrangements was:

- translocations - 1.47% (10)
- inversions - 1.76% (12)
- numerical chromosomal abnormalities - 0.44% (3)

Conclusions: partners of infertile couples requiring IVF or ICSI treatment appear to be affected by higher frequency of chromosomal rearrangements than the general population.

#### P04.10

##### The influence of consanguinity on miscarriage

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Comparative analysis of consanguinity in families with miscarriage was been carried out. The first group of families comprised those with miscarriage before 12 weeks of pregnancy (532 families) was subdivided into 3 sub-groups: 1 miscarriage - 249 families; 2 miscarriages - 210 families; and 3 or more miscarriages - 73 families. The second sample included 862 families who also had a history of live-births, abdominal pregnancies, and medical abortions) and miscarriages up to 28 weeks of pregnancy. This was subdivided into 2 sub-groups: the sub-group with 1 miscarriage - 391 families; the one with 2 and more miscarriages - 471 families. The control group included 272 families who had no history of miscarriage. The findings showed that in the families with 3 and more miscarriages before 12 weeks of pregnancy inbreeding was more likely. This may lead, in case of successful pregnancy, to an increase in the frequency of births with genetic disorders.

#### P04.11

##### Multiplex single base extension analysis for the detection of thrombophilia and folate related genes mutations in women with miscarriages

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Miscarriage is a significant clinical problem with many etiologies. Certain thrombophilia gene mutations have been associated with an increased risk for recurrent miscarriages. Chromosome aneuploidy is the most common cause of miscarriage. Mutations in folate related genes can lead to DNA hypomethylation and abnormal chromosomal segregation during meiosis (non-disjunction). Thus, mutations in folate genes might represent a risk factor for having a fetus with chromosomal aneuploidy. We have developed a multiplex single base extension reaction assay that allows analysis of 10 different mutations in thrombophilia and folate related genes (Factor V Leiden G1691A, Factor V A1299G, Factor II G20210A, Factor XIII G34C, PAI-I -675 4G/5G, MTHFR C677T, MTHFR A1298C, MTR A2756G, MTRR A66G and FG-β -455G/A) in one single reaction. The PCR primers have been designed to give different fragment sizes (129 to 420 bp), allowing their separation by polyacrylamide gel electrophoresis. Different length of poly (dC) tails was attached to the single base extension primers to allow analysis of all 10 mutations by one multiplex SNaPshot analysis. The SNaPshot fragments were separated by capillary electrophoresis. Using this method we have successfully studied 200 patients with spontaneous abortions (138 Slovenian, 34 Albanian 28 Macedonian women) and 208 controls (130 Slovenian, 38 Albanian and 40 Macedonian women). In conclusion, we have developed a rapid, simple, reliable and inexpensive method for determination of 10 mutations in thrombophilia and folate related genes that might be implicated in the etiopathogenesis of miscarriages.

#### P04.12

##### Genetic selection? Interaction between MTHFR and TYMS polymorphisms may affect survival *in utero*

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Genetic variation in folate metabolism has been associated with survival *in utero*, the success of *in vitro* fertilisation, multiple pathologies and longevity. We have looked at the prevalence of genetic variants of the enzymes MTHFR and TYMS in 2656 DNA samples derived from five cohorts collected in Norfolk and Cumbria. The simultaneous analysis of genetic variants of the *MTHFR* and *TYMS* loci was carried out to investigate a putative gene-gene interaction that was initially observed in an elderly male population from Norfolk.

In five separate population cohorts we have demonstrated that the proportion of individuals who are homozygous for the 2R allele of the 5'UTR *TYMS* polymorphism is less in individuals who are homozygous for the T allele of *MTHFR* 677 than in individuals homozygous for the C allele of *MTHFR* 677 ( $p = 0.02$ ).

The mean ages of our cohorts varied from 27 to 92 and so the consistent observation can not be due to an age-related survival factor.

These data provide evidence for a gene-gene interaction and are suggestive of genetic selection *in utero*, with some pregnancies more or less viable because of genetic variation. The MTHFR and TYMS enzymes compete for limiting supplies of folate required for homocysteine methylation. Given the established role of folate in early development, this evidence suggests that fetal viability may be influenced by maternal folic acid intake and /or genotype.

#### P04.13

##### Genetic factors related to the development of uterine leiomyoma during pregnancy

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Uterine leiomyoma (UL), a common hormonal-dependent benign neoplasm, is thought to affect up to 40% of women during their lifetime. It often leads to fetal wastage as the lesion undergoes a rapid expansion during pregnancy. Identification of genetic factors that predispose to UL formation could provide further insight into the trigger mechanism and risk factors leading to its development.

We examined the distribution of common polymorphisms in ERα (Thr397Cys and Cys351Gly), IGF-II (C/T 820), p53 (Arg72Pro), CYP2A13 (Arg257Cys), XRCC1 (Arg399Gln) and AR (CAG repeat) genes among 146 Bulgarian women: 72 healthy pregnant controls and 74 women affected by UL during pregnancy. Genotypes were determined by PCR - RFLP.

The case-control analysis showed no significant difference in the distribution of IGF-II, p53, CYP2A13, XRCC1 and AR variant polymorphisms among patient and control groups.

ERα polymorphisms Thr397Cys and Cys351Gly showed a statistically significant association to leiomyoma development during pregnancy. The distribution of Thr397Cys genotypes in control subjects was as follows: 23,9% p/p (+/+) 44,8% p/P (+/-) and 31,3% P/P (-/-), while in UL patients it was: 37,3% p/p (+/+) 44,8% p/P (+/-) and 17,9% P/P (-/-), ( $P < 0.03$ ). For the Cys351Gly polymorphism, genotype distribution was 30,9% x/x (+/+) 38,2% x/X (+/-) and 30,9% X/X (-/-) among controls, and 48,6% x/x (+/+) 38,6% x/X (+/-) and 12,8% X/X (-/-) among leiomyoma patients ( $P < 0.005$ ).

We conclude that the ERα polymorphisms Thr397Cys and Cys351Gly are implicated in pregnancy - related leiomyoma.

#### P04.14

##### Is NF-κB inhibitor alpha (IκBa) involved into oocyte-to-embryo transition?

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NF-κB activity is regulated by interaction with inhibitory proteins (IκB). The canonical p65/p50 heterodimer is bound to IκBα that inhibits the NF-κB activity by masking its NLS domain and so blocking NF-κB ability to bind DNA. NF-κB-IκBα complex is continuously shuttling between nucleus and cytoplasm. Its nuclear export rate exceeds its import rate and thus the complex is generally cytoplasmic. NF-κB signalling studies during oocyte maturation have been limited. The upregulated IκBα expression has been observed in murine oocyte aging suggesting that

NF- $\kappa$ B pathway deregulation may be related to oocyte competence. In male gamete development, NF- $\kappa$ B is activated in pachitene stage and in the following germinal differentiation steps triggering specialized genes expression.

We analysed *IκBα* transcript levels in meiotic arrested bovine oocytes at different stages of maturation (diplotene of prophase I and metaphase II) and in early embryogenesis. We included three expressed oocyte genes: *BMP15*, *GDF9*, *SPIN1*. We examined *ZAR1*, specific oocyte maturation marker, whose transcript generally underwent degradation during meiosis. We observed a significant reduction of the *IκBα* mRNA during meiotic maturation. On the contrary, we observed that the amount of *IκBα* protein increased during the transition from immature to mature oocytes, while it was significantly decreased in embryos. Further analysis will be performed to establish how *IκBα* is regulated during developmental competence and if it is involved in human diseases such as premature aging and infertility.

#### P04.15

##### Bench to Bedside: Translating genetic eye research into different reproductive options.

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Purpose: To demonstrate the translation of genetic eye research into the IVF (In Vitro Fertilisation) and preimplantation genetic diagnosis (PGD) setting for a family with Autosomal Dominant Optic Atrophy (ADOA).

Methods: Genetics is rapidly impacting on clinical care. It helps confirm the clinical diagnosis, as well as identify carrier status and mutations in non-symptomatic patients who have known familial mutations. This helps to facilitate early diagnosis and subsequent interventions. The clinical need and cost effective benefits of genetic testing in ophthalmology has already been well documented in retinoblastoma RB1 testing. While many genes have been identified as causing inherited ocular disease, genetic testing has primarily been performed on a research basis. However this knowledge from the laboratory is translating into clinical care and providing couples with reproductive options in the form of prenatal diagnosis (PND) and pre-implantation genetic diagnosis (PGD). We present a family identified with a known OPA1 mutation as part of the Autosomal Dominant Optic Atrophy research study who have chosen to utilise In Vitro Fertilisation coupled with pre-implantation genetic diagnosis to avoid passing this condition on to their child.

Results: This couple elected to undergo two IVF/PGD cycles at Monash IVF. They achieved a successful singleton pregnancy in their second cycle and have subsequently given birth to a healthy baby girl.

Conclusions: From research studies we are often able to provide individuals with information on their specific genetic mutation and some couples choose to use this knowledge to broaden their reproductive options.

#### P04.16

##### Vascular Endothelial Growth Factor Gene Polymorphism and Ovarian hyperstimulation syndrome

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Purpose : Ovarian hyperstimulation syndrome (OHSS) is one of the most important complication of assisted reproduction treatment . The pathophysiology of OHSS remains to be full elucidated. Many substances and more recently, vascular endothelial growth factor (VEGF) have been suggested to be involved in the pathogenesis of OHSS . VEGF is a member of a family of heparin - binding proteins that act directly on endothelial cells to induce proliferation and angiogenesis . In vivo , VEGF is a powerful mediator of vessel permeability, Increased vascular permeability mediated by VEGF has been implicated in the sudden increase in capillary permeability , then it is logical to examine the relationship between the VEGF polymorphism and OHSS. In this paper, we conducted a case - control study to evaluate potential as-

sociation between OHSS and VEGF gene 460 polymorphism.

Methods : 75 OHSS patients and 85 normoresponse patients were enrolled in this study. Polymerase chain reaction- restriction fragment length polymorphism analysis was used to resolve the VEGF 460 genotype of OHSS patients and normoresponder controls.

Results : The frequency of homozygosity of the VEGF 460 gene was significantly higher among women with OHSS.

Conclusion : Homozigosity of the VEGF 460 gene may serve as a susceptibility factor affecting for OHSS.

#### P04.17

##### Prevalence of epimutations in imprinted *PLAGL1* (*LOT1/ZAC1*) locus in first-trimester miscarriages

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Genomic imprinting plays a critical role in regulation of fetal development. Previously we have reported a tissue-specific loss of methylation in *KCNQ1OT1* (11p15) in 9.5% of spontaneous abortions, but normal epigenetic status of *H19*, *CDKN1C* and *SNRPN* genes. The aim of the present research was investigation of differential methylation of maternal imprinted gene *PLAGL1* (6q24-25), which is involved in control of cell proliferation. Methylation-specific PCR of *PLAGL1* promoter was performed using DNA from extraembryonary mesoderm (EM) and cytotrophoblast (CT) of 87 first-trimester missed abortions. Thirty induced abortions were studied as a control group. Nine miscarriages (10.3%) have revealed a loss of methylation of *PLAGL1* on maternal chromosome. Loss of imprinting (LOI) was confined by EM or CT in 6 and 2 miscarriages, respectively. For one embryo with epimutation only cytotrophoblast was available. Two conceptions have revealed LOI in both *PLAGL1* and *KCNQ1OT1* genes confined by EM. Tissue-specificity of epimutations allows suggesting independent sporadic epigenetic events in different embryonic germ layers after its divergence. Importantly, significant prevalence of recurrent pregnancy loss was found in women having a miscarriage with *PLAGL1* epimutation in compare with women having a conception with normal imprinting (33.3% vs 7.7%, respectively, P=0.05). At the same time, no significant differences in the maternal age were found between these groups. Our results provide evidence that errors of imprinting maintaining mechanisms on maternal chromosomes during embryo development may be among molecular processes responsible for dysfunction of imprinted loci and recurrent pregnancy loss.

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#### P04.18

##### Common variants on chromosome 9p21 are associated with preeclampsia in the Finnish population

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Preeclampsia is a pregnancy specific disorder, characterized by hypertension and proteinuria. Its etiology and pathophysiology remain poorly understood, but it has clearly a familial component, suggesting that genetic factors play a role in the susceptibility. We have previously reported linkage to preeclampsia on chromosome 9p13. Interestingly, several genome-wide association (GWA) studies have identified a region on chromosome 9p21 that is associated with coronary artery disease (CAD) and type 2 diabetes (T2D). As preeclampsia has been linked to increased risk for both T2D and CAD later in life, and our linkage region is closely located to the GWA signals, we decided to genotype previously reported associated SNPs in 15 preeclampsia families and a nation-wide case-control preeclampsia cohort (340 cases and 357 matched controls). In total, 23 markers were successfully genotyped. Single-marker and haplotype associations were calculated and four SNPs showed nominal association (P<0.02) in the case-control cohort when women with gestational hypertension were excluded. When the

two cohorts were combined, the odds ratio for the risk haplotype was 1.38 (1.11 - 1.71). In order to increase power, we included genotypes for three out of the four SNPs from 260 additional controls representing a Finnish population-wide sample set and observed significant association for two SNPs (corrected  $P=0.02$  and 0.04). These findings were tested in a UK case-control preeclampsia sample set (237 cases and 200 controls), but no association was observed. In conclusion, these results indicate that chromosome 9p21 confers risk for development of preeclampsia in the Finnish, but not in the UK population.

#### P04.19

#### Polymorphism of factor V Leiden, prothrombin and endothelial nitric oxide synthase genes in development of preeclampsia

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Preeclampsia (PE) is still one of the leading causes of maternal and fetal morbidity and mortality. Recently, significant associations of factor V Leiden (FV Leiden), prothrombin (FII) and endothelial nitric oxide synthase (eNOS) genes polymorphism with thrombophilia at pregnant, recurrent spontaneous miscarriages and preeclampsia in women of different populations were reported (Wilson M. et al, 2002; Kujovich J., 2004). These loci are located on different chromosomes and encode products involved into various metabolic pathways leading to obstetrics pathology. Therefore, we studied the eNOS VNTR polymorphism in 4 introne, FV Leiden G1691A polymorphism and FII G20210A polymorphism in women with PE from Bashkortostan (Russia). DNA from 198 preeclamptic pregnant women and from 206 healthy control pregnant women were genotyped for polymorphisms using PCR technique and subsequent enzyme digestion. Allelic and genotypic frequencies of eNOS polymorphism and FII polymorphism did not differ between both groups of pregnant women. A significant difference were observed in FV Leiden genotype and allele frequencies between preeclamptic pregnant women and healthy pregnant women. Factor V Leiden was associated with PE (OR=3.22).

#### P04.20

#### BMP15 gene molecular analysis in XX premature ovarian failure women

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Premature ovarian failure results from accelerated reduction of the oocyte pool and a consecutive loss of granulosa cells. Therefore, genes expressed in oocytes and promoting the proliferation of granulosa cells are strong candidates for POF. Bone morphogenetic protein-15 (BMP15) gene at Xp11.2 and its autosomal parologue GDF9 (growth differentiation factor-9: GDF9) fulfill these criteria. Recently, heterozygous mutations in BMP15 gene have been identified as a possible cause of ovarian failure.

The objective of our study was to verify the involvement of BMP15 variations in a cohort of 17 Tunisian patients who were diagnosed as POF women and for who a 46,XX karyotype was identified.

Seventeen women were referred to our cytogenetic laboratory of Pasteur institute with the diagnosis of POF. Patients with POF as a result of surgery, radiation, chemotherapy, or chromosome aberrations were not included in this study.

The entire coding sequence and intron-exon junctions of BMP15 gene were analyzed in all patients. Patients' samples were screened by direct sequencing.

Genetic analysis revealed the presence of three variants of BMP15 gene in three of 17 POF patients: SNP1: rs3810682, SNP 2: rs41308602 and SNP 3: rs17003221.

Our results will be compared with literature findings.

#### P04.21

#### Cytogenetic study in premature ovarian failure

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Premature Ovarian Failure (POF) is defined as the occurrence of menopause before the age of 40 years, biochemically low levels of gonadal hormones (estrogen & inhibin) and high levels of gonadotrophins (FSH & LH). POF is a heterogeneous disorder affecting approximately 1% of women <40 years, 1:10,000 women by age 20 and 1:1000 women by age of 30. Several causes of POF have been identified, including X chromosome aberrations. This study finds out the proportion of genetic causes of POF and analyze different chromosomal pattern. Fifty patients of POF were referred for cytogenetic analysis. Family history, age, occupation, disease information and all other medical records were reviewed. Blood was collected and lymphocyte cultures were set up. Twenty GTG banded metaphases were analysed for chromosome complement. Mean age and height was found to be 20.2 years and 150cm respectively. FSH level was found to be >40mIU/ml in all patients. 82% of cases had a normal karyotype. In 6% cases karyotype was 45,XO whereas 16% of the cases showed mosaicism with normal cell lines. Two patients had a chromosomal constitution 46,X,i(X) whereas other two had the karyotype 46,XX,del Xq(13.3-21.1) and 46,XX,delXq(22-24). Various reports suggested that X chromosome deletions associated with POF are more common than translocations. In this study X chromosome aberration are involved in all cases as reported in the earlier studies. This mosaicism could explain an accelerated loss of gametes in the ovary. Cytogenetic analysis in association with molecular techniques could allow finding the molecular pathogenesis of the ovarian failure.

#### P04.22

#### Clinical, hormonal and cytogenetic study in Premature Ovarian Failure (POF)

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POF is clinically presented with complaints of abnormal menarche, irregular menstruation, anxiety, depression, psychological distress and was less satisfied with their sexual life. POF is believed to be major cause of infertility in women. Several causes of POF have been identified, including X chromosome aberrations. This study correlates the clinical, hormonal and cytogenetic finding causes of POF. This study included 50 patients with POF. Detailed history, age, occupation, disease information and all other medical records were reviewed and recorded. 5 ml blood was collected and lymphocyte cultures were set up. Twenty GTG banded metaphases were analysed for identifying chromosomal abnormality. Mean age and height was found to be 20.2 years and 150 cm respectively. In this study 6 % had 45 XO chromosome complement and 16 % were found to be mosaic. In cases with structural abnormality, FSH level were  $52.4 \pm 8.1$  mIU/ml in all patients (normal  $32.4 \pm 4.5$  mIU/ml). Two patients had chromosomal constitution 46, X, i(X) whereas other two had 46,XX,delXq(13.3-21.1) and 46,XX,delXq(22-24) complement. Various studies have suggested that X chromosome deletions associated with POF are more common than translocations. The critical requirement for ovarian local and long arm of X chromosome the structure for intact X chromosome is required for normal ovarian function. In this study X chromosome aberration are involved in cases as reported in the earlier studies. This mosaicism could explain an accelerated loss of gametes in the ovary. Cytogenetic analysis in association with molecular techniques could allow finding the molecular pathogenesis of the ovarian failure.

**P04.23****Results of prenatal cytogenetic and molecular genetic study of the high risk chromosomal or monogenic diseases patients**

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Results of 162 prenatal diagnostics have been analysed. 144 prenatal cytogenetic studies have been performed. In 20 amniocyte cultures numeral (8 cases) and structural (12 cases) changes of the karyotype were detected. In 10 cases chromosomal rearrangements arise due to translocations in parents. Balanced chromosomal translocations were detected in 8 amniocyte cultures. Robertsonian translocations were found in 5 cases: 14;21 – 3 cases, 13;15 and 13;22 – one case each. In 11 of cases, imbalanced karyotype was diagnosed: trisomy of 21-st and 18-th chromosome; simple and mosaic forms of gonosomal monosomy; additional marker chromosomes; mosaicism 46,XX/92,XXXX (30:20). Non-balanced derivative chromosomes were detected in two foetuses – 46,XY,der(4),t(4;7)(q35;q31.1)mat and 46,XY,der(10),t(10;7)(q26.13; p21.2)pat.

Prenatal molecular genetic diagnostics of families with heterozygous mutations carriers, whose mutations lead to cystic fibrosis and Nijmegen syndrome, was performed. DNA was extracted from cells of amniotic fluids or chorion.

13 prenatal diagnostics of Cystic Fibrosis in families of heterozygous carriers of CFTR gene mutations have been performed. As a result, following foetus genotypes were identified: F508del/F508del - 3, F508del/1717-1G>A - 1, F508del/wt - 6, N1303K/wt - 1, wt/wt - 2. 5 prenatal molecular genetic diagnostics of Nijmegen breakage syndrome in families of heterozygous carriers of 657del5 mutation of NBN gene were performed. 2 foetuses were homozygous for 657del5 mutation of the NBN gene (genotype 657del5/657del5) and 3 cases -heterozygous for this mutation (genotype 657del5/ wt). The prenatal conformation of monogenic or chromosomal diseases mostly caused termination of pregnancy by decision of family.

**P04.24****Immunogenetic Markers of Secondary Infertility**

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In marital couples with secondary infertility and multiple first trimester miscarriages, HLA-antigen distribution in A-, B-loci and a single nucleotide polymorphism (SNP 1082 G>A) of the promoter region of IL-10 were studied. 157 individuals with secondary infertility with 2 or more involuntary first trimester miscarriages and 64 individuals with primary infertility were examined. The control group included 227 healthy individuals with healthy children. It was determined that immunogenetic markers of secondary infertility were HLA-antigens A10, B41 and B38, with primary infertility associated with B7 and A19. The results also show that more than 50% investigated couples had homologous HLA genotypes. We studied the SNP1082 G>A of the IL-10 promoter region in 50 couples with secondary infertility and recurrent loss of first trimester pregnancies. We determined an allele A (low expressing allele) frequency of 38.2%, and a G allele (high expressing) frequency of 61.8%. We observed a statistically significant, compared to the control group, increase of high expression GG-genotype frequency, and a statistically significant decrease of normal expression IL-10 gene (AG-genotype) frequency. This suggests that IL-10 is active in the pathogenesis of recurrent miscarriages. It was confirmed that the presence of common HLA-antigens in marital couples and an increase of high expression associated GG-genotypes at SNP -1082 G>A pil-10 were prognostically unfavourable for completion of pregnancy.

**P04.25****Couples with recurrent spontaneous abortions in Moldova (genetic characteristics).**

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Infertility and spontaneous abortions are a major medical-social problem in the conditions of demographic crisis existing in the Moldova during the last decade. It's claimed that all couples with reproductive problems needs complex investigations which include medico-genetics

analysis.

**Materials and methods.** In this study, 49 couples with miscarriage passed medico-genetic counseling with cytogenetic and molecular genetic analyses. Cytogenetic features of peripheral blood lymphocytes cultivated according to standard techniques and DNA analyses to estimate the differential risk associated with DQ genotypes.

**Results.** Analyses of family history revealed what in 67.39% of couples were cases of recurrent spontaneous abortions (RSA), in 30.43% of couples were cases of RSA with congenital anomalies, and in 2.17% RSA and single-gene disorders. The analysis of TORCH-infection revealed that test for serum IgG were positive in 45.13% women with RSA, in 42.86% women with RSA and congenital anomalies. Cytogenetic analysis showed different chromosomal aberrations involved 1, 9, 15, 17, and 22 chromosomes in 8% of cases. Were investigated 32 persons (total 64 chromosomes) by molecular analysis of HLA haplotypes DQA1 and DQB1, discovered prevailed haplotypes of DQA1\*0101/0102 (35.9%) as well as DQA1\*0501 (43.6%) in group of patients and in control group. Haplotype DQB1 \*0201 (26.6%) was more frequent in patients as well as in control group (25.0%).

**Conclusions.** These data indicated that medico-genetic counseling with cytogenetic and molecular genetic investigations may be indicative in diagnosis of causes of RSA. Cytogenetic analysis could be valuable for these couples when clinical data fail to clarify the cause.

**P04.26****Role of mitochondria in repeated pregnancy loss**

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**Introduction:** Mitochondria are small structures in cells that generate energy for the cell to use. All mitochondria are inherited from the mother's ovum. About 1 in 300 couples involve with Repeated Pregnancy Loss (RPL) and the main part of them remains unknown. The aberrant expression of apoptotic related genes is seen in RPL. It seems internal apoptotic pathway and mitochondria have important role in fertilization and proliferation of the cells.

**Methods:** In total 96 females who were suffered from idiopathic RPL. Four multiplex PCR are done on each sample for detection of deletions. D-loop part is analyzed by PCR-sequencing method. Bax and Bcl2 genes is evaluated by PCR-sequencing method for promoter regions and PCR-SSCP for exones.

**Results:** No deletions were found in 96 DNA samples. Mononucleotide repeat (poly C) from 303 to 315 nucleotide positions (D310) exhibited a polymorphic length variation (among 89 cases; 7C in 43, 8C in 34, 9C in 8, and ≥10C in 4 females. Many sequence alterations identified in D-loop region of cases, that will be described as mtDNA haplogroups or novel nucleotide variants. Nucleotide change in Bax gene was seen in promoter region at -55 A>G.

**Discussion:** Some of these nucleotide alterations might be involved in RPL and could be included in a panel of molecular biomarkers for susceptibility in pregnancy loss and even failure of in-vitro fertilization. We believe that mutation in Bax and Bcl2 genes will lead to early apoptosis. The results can be used in assessment of RPL.

**P04.27****Blood pressure in 8 and 10- year-old singleton ICSI children**

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**Introduction:** To evaluate if the in-vitro procedure in humans has long-term consequences on the cardiovascular functioning, longitudinal blood pressure measurements were compared between children born after intra-cytoplasmic sperm injection (ICSI) and after spontaneous conception (SC).

**Material and Methods:** Longitudinal questionnaire data and parameters of physical examination of 8-year-old ICSI children were compared with results of peers born after SC. At the age of 10 years, 108 of the initial recruited 150 ICSI children were re-examined and 93 out of

the 147 SC children. All children were singletons born after 32 weeks gestation.

Results: Height and weight in ICSI and SC children were comparable at the age of 8 and 10 years. Systolic and diastolic blood pressure were higher in ICSI than in SC children at the age of 8 years (98 mmHg versus 94 mmHg;  $p<0.001$  and 59 mmHg versus 55 mmHg;  $p=0.001$  respectively). The difference remained after correcting for birth characteristics, maternal factors and current physical characteristics. Ten-year-old ICSI children had a comparable systolic but a lower diastolic blood pressure compared to their spontaneous conceived peers (99 mmHg versus 99 mmHg;  $p=0.5$  and 65 mmHg versus 68 mmHg;  $p=0.002$  respectively), although the effect of lower diastolic blood pressure attenuated after adjusting for confounders.

Conclusion: ICS is associated with a 5 mmHg increase in blood pressure at 8 years, but this could not be confirmed at age 10. Our findings warrant long-term follow-up of ICSI conceived individuals to assess possible effects of periconception events on cardiovascular health in later life.

#### P04.28

#### Detoxification and blood coagulation system genes polymorphisms: Possible involvement in Recurrent Pregnancy Loss

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Recurrent Pregnancy Loss (RPL) represents an intriguing problem in obstetric practice in which genetic factors play a role. Enzymes such as P4501A1 (CYP1A1) metabolize organic compounds to reactive compounds which damage cells and DNA. N-acetyltransferase 2 (NAT2) is involved in the biotransformation metabolism of aromatic amines. Glutathione S-transferase (GST) catalyze the binding of a large variety of electrophils to the sulphydryl group of glutathione. During pregnancy, changes in blood coagulation may play a role in the occurrence of abortion. Factor V Leiden (FVL) gene, is associated with a hypercoagulable state and increased susceptibility for venous thrombosis. Factor II (prothrombin) gene is associated with higher plasma prothrombin concentrations. Aim of this study was to investigate the possible role of I and II stage detoxification and coagulation systems genes polymorphisms in the pathogenesis of RPL. The polymorphic variants of those genes were analyzed in 24 women (case group) with RPL and in 171 women (control group) with the uncomplicated obstetric history. The frequency (80%) of NAT2 gene SS genotype in case group was significantly ( $p<0.05$ ) higher than in control group (57%). Frequencies of GSTM1, GSTT1, CYP1A1, FII and FVL polymorphic variants were practically similar in both analyzed groups. It had been shown that NAT2 S/S genotype really can be involved in the process of RPL, which may be associated with changes in steroid hormones level. From our data the identification of NAT2 S/S genotype can be used as a marker for high risk recurrent pregnancy loss prediction in genetic testing family programs.

#### P04.29

#### Unsymmetrical X-inactivation and loss of methylation of IGF2/H19 imprinting center in spontaneous abortions with trisomy 16

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Skewed sex ratio (0.35-0.45) as well as unsymmetrical X-chromosome inactivation (XCI) in prenatally diagnosed fetuses with trisomy 16 mosaicism provides evidence for differential survival of females with this aneuploidy. There seems to be tentative autosomal trans-factor(s) associated with chromosome 16 and can control XCI. CCCTC-binding factor (CTCF) potentially can be the one. It is localized on chromosome 16, has binding sites in choice and imprinting center (IC) of X chromosome and in IGF2/H19 IC. So, we suggest that overdosage of CTCF may induce hypomethylation of some IC. Firstly, we studied the level of trisomic cells in extraembryonic mesoderm (EM) and cytotrophoblast (CT) of 28 spontaneous abortions (SA) with cytogenetically verified trisomy 16 using FISH. XCI and methylation of IGF2/H19 IC were analyzed in the same samples. Sex ratio among SA with mosaic karyotype (<90% of aneuploid cells) appears to be skewed with prevalence of male abortions (1.25) while females prevail among non-mosaic embryos (0.43). At the same time, unsymmetrical XCI in EM was

observed in 66% of embryos with more than 80% of trisomic cells. Hypomethylation of IGF2/H19 IC was found in most of analyzed cases (5 out of 6) and was predominantly associated with CT with high level of mosaicism (>74%). Our results provide the first evidence for the possible linkage of tentative XCI trans-acting factor to chromosome 16. Hypomethylation of IGF2/H19 IC in extraembryonic tissues of embryos with trisomy 16 mosaicism can be the other proof of the existence of such factor and its linkage to chromosome 16.

#### P04.30

#### Comparison of PRM1 and PRM2 genes polymorphisms in fertile Czech and normozoospermic German men

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Data in German males provide evidence that PRM1 c.230A>C and PRM2 c.373C>A are significantly associated with reduced sperm numbers (Tüttelmann, unpublished). The aim of study was to compare the prevalence of PRM1 and PRM2 polymorphisms of Czech and German men to disclose the impact of different ethnical and life style pattern on impaired spermatogenesis .

PRM1 and PRM2 sequencing was performed in 99 and 94 Czech men with verified fertility and in 77 and 73 German normozoospermic men. BigDye Terminator chemistry was used on ABI 3130xl Genetic Analyzer.

In PRM1 gene one rare SNP c.102G>T (rs35576928) was detected only in 1.3 % Germans. One rare (c.54G>A, rs35262993) and one common SNP (c.230A>C, rs737008) in PRM1 gene were found with same allele and genotype frequencies. No mutations were found.

In PRM2 gene three rare SNPs c.300A>G, c.281C>T and c.290C>T were not found in Czech males. Two common SNPs (c.298G>C, rs1646022 and c.373C>A, rs2070923) were identified with identical allele and genotype frequencies.

Despite the ethnical difference the allele prevalences of the most frequent PRM1 and PRM2 polymorphisms are identical in Czech fertile and German normozoospermic males, except the four rare PRM1 and PRM2 SNPs present only in German males. Homozygosity of PRM1 230C and PRM2 373A might be associated with reduced sperm numbers also in Czech subfertile males.

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Clinically important PRM1 and PRM2 polymorphisms in Czech fertile and German normozoospermic males				
Gene	Polymorphism	Czechs	Germans	P
PRM1	230A>C	A frequency	70.7% (140)	71.4 % (110)
		C frequency	29.3% (58)	28,6 % (44)
		AA	48.5% (48)	53.2% (41)
		AC	44.4% (44)	36,4% (28)
		CC	7.1% (7)	10,4% (8)
PRM2	373C>A	C frequency	69.7% (131)	69,9% (102)
		A frequency	30.3% (57)	30,1% (44)
		CC	47.9% (45)	52.0% (38)
		AC	43.6% (41)	35.6% (26)
		AA	8.5% (8)	12.4% (9)

#### P04.31

#### The role genetic factor in symphysis pubis dysfunctions

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Recent studies have shown that genetic effects on developing osteoporosis. The pregnant women with dysfunction symphysis pubis have a greater risk of preclinical osteoporosis.

Objective. To determine the genetic effects of vitamin D receptor (VDR), collagen type I (ColI1A1) genes on development of symphysis pubis dysfunctions by pregnant of Kazakh populations.

Subjects. The material of the study was DNA of 100 health (a control group) and 50 pregnant with rupture of the symphysis (a basic group).

Results. The frequency of favorable TT genotype of VDR gene among the control group was  $62,0 \pm 4,87\%$ , in the basic group statistically lower -  $38,0 \pm 6,93\%$  ( $\chi^2=7,73$ ;  $p<0,05$ ). We revealed that the frequency of unfavorable CC genotype is significantly more common in basic group ( $8,0 \pm 3,87\%$ ) than in control subjects ( $1,0\%$ ) ( $\chi^2=5,07$ ;  $p<0,05$ ).

The distribution of genotype frequencies differed significantly between patients basic group and controls, with the GG genotype of Coll1A1 occurring more frequently in the controls ( $26.0 \pm 6.26\%$  and  $62.0 \pm 4.87\%$  respectively;  $\chi^2=17.28$ ;  $p<0.001$ ). We found a significantly higher prevalence of the unfavorable TT genotype in the patients compared to the controls ( $14 \pm 4.95\%$  and  $3 \pm 1.7\%$ , respectively) ( $\chi^2=6.48$ ;  $p<0.01$ ). Conclusion. We conclude that a significant association exists between the VDR and Coll1A1 genes polymorphisms and symphysis pubis dysfunctions and indicates the importance of this genetic characteristic as a marker for increased development of symphysis pubis dysfunctions risk.

#### P04.32

#### Telomere length in human preimplantation embryos and its correlation with chromosomal abnormalities and maternal age

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Telomeres are TTAGGG repeats at the end of chromosomes. Telomeres get shorter during cell division contributing to cellular senescence. Telomere length is set to a maximum during early oogenesis. One theory supports that telomere shortening may be mediated in late exit from the fetal production line and long interval ovulation in the adult thus causing reproductive ageing in women. Excessive telomere shortening causes chromosome misalignment during meiosis.

In this study, telomere length was measured in embryos on day 5 of preimplantation development and correlated to chromosomal ploidy and morphology.

Embryos were donated from patients undergoing treatment in the assisted conception unit. Seven couples, generating 40 embryos consisting of 11111 cells took part. Quantitative fluorescent in situ hybridisation (FISH) measured the average telomere length of every cell using a pan-telomeric probe. Conventional interphase FISH on chromosomes 13, 15, 16, 18, 21 and 22 was used to assess aneuploidy. Reproductive history of the couples and embryo morphology was taken into account.

Chromosomally abnormal embryonic cells had significantly shorter telomeres than chromosomally normal cells (p38 years) were significantly shorter than those in younger women ( $p<0.05$ ). Significantly shorter telomeres were found in all AMA derived embryos irrespective of chromosomal content, in agreement with the telomere theory of reproductive ageing.

Telomeres play an important role in cell division and shorter telomeres could possibly affect embryonic mosaicism, quality and survival; all vital for preimplantation embryo development.

#### P04.33

#### Use of Y chromosome specific repeat sequencing for sexing in cattle

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Embryo sexing is one of the important ways for sex selection of offspring. This is a potential method to considerably improve animal breeding and the efficiency of dairy and meat production. a novel repeated sequence specific to male cattle has been identified and named S4 . S4 is a 1/5 Kb repeating unit contains various internal repeated sequence. S4 is localized on long arm of the Y chromosome in the region near to ZFY genes. Aim: The objective of this study was to establish a simple, sensitive, reliable, reproducible and cost effective PCR based technique for sexing.. Materials and Methods: Genomic DNA was extracted from the whole blood samples of 4 male and female cattles. PCR and real time PCR were performed using specific primers for this region. Result: By this PCR based methods we could differentiate between female and male genomic DNA. Real time PCR employed in order to have a quantitative method. Discussion:With this technique we can distinct male from female using as much as 1pg DNA . Using this method we could determine the sex of an embryo (4 blastomers) . The Real time PCR method optimized here was able to be used for the quantitative detection of Y chromosomes in semen.

#### P05. Prenatal and perinatal genetics

##### P05.01

##### Prenatal diagnosis of 5p deletion syndrome following abnormally low maternal alpha fetoprotein

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Here we report prenatal diagnosis of 5p deletion syndrome. Amniocentesis was performed following an abnormally low measurement of a screening of serum alpha fetoprotein. The triple test result was in favour of Down syndrome. However, the karyotype showed a terminal deletion of the short arm of chromosome 5 including the critical region 5p15 for cri du chat syndrome. The parents had normal karyotypes. To our best knowledge this is the first reported case of prenatal detection of -5p syndrome following abnormal alpha fetoprotein level. It is possible that the abnormally low measurement of a screening of serum alpha fetoprotein may be a marker for cri du chat syndrome

##### P05.02

##### The utility of maternal serum ADAM12 in first and second trimester aneuploidy screening

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The aim of the study was to estimate the utility of ADAM12 as aneuploidy marker for 1st and 2nd trimester.

ADAM12 was measured by DELFIA ADAM12 Research Kit (Perkin Elmer). The control 11-17th week levels were ascertained in frozen (-20 °C) maternal sera from screening within years 2001-2008. For each day at least 30 samples were used for control percentile (P) calculations. The 54 1st and 11nd trimester sera from pregnancies with different types of chromosomal aberrations were tested to ascertain the degree of deviation from P50.

The P50 levels within 11-17th week raised from 456 to 1133 µg/l. The highest 1st trimester prevalence of trisomy 21 is within P25-50, within 11nd P50-75. The 2/5 cases with trisomy 18 are in the range P<5-10. The 3/3 cases with trisomy 13 are under P25. All triploidy cases are below P5. The 5/6 cases with 47,XXX are under P5, 1/6 is below P25. All 47,XXY and 47,XYY are in P50-95 range. The 5/11 cases with Turner syndrome (including mosaicism) were within P25-50, 3/11 in P75-95. In four cases with structural chromosomal aberrations and in one mosaic trisomy 10, the levels were under P25. Decreased ADAM12 levels were in 42.3 % of studied aberrations. Our results support further ADAM12 studies to determine its additional value for 1st and 11nd trimester screening.

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Trimester 1st	Aneuploidy	Percentile distribution of aneuploidies								Total
		<5	5-10	10-25	25-50	50-75	75-90	90-95	>95	
1st	+21	-	1	1	4	1	-	-	1	8
	+18	1	-	-	1	2	-	-	-	4
	+13	1	-	1	-	-	-	-	-	2
	Triploidy	5	1	-	-	-	-	-	-	6
	47,XXY	-	-	-	-	1	-	-	-	1
	47,XXX	2	-	1	-	1	-	-	-	4
	47,XYY	-	-	-	-	-	1	1	-	2
	45,X	-	-	-	3	4	-	1	-	8
2nd	+21	-	-	2	2	4	-	-	1	9
	+18	-	1	-	-	-	-	-	-	1
	+13	-	-	1	-	-	-	-	-	1
	Triploidy	2	-	-	-	-	-	-	-	2
	47,XXY	-	-	-	-	1	-	-	-	1
	47,XXX	2	-	-	-	-	-	-	-	2
	47,XYY	-	-	-	-	-	-	-	-	0
	45,X	-	-	-	2	-	1	-	-	3
Total		22 (40.74%)				26 (48.15%)	6 (11.11%)			54

**P05.03****Application of genome wide 250k SNP array analysis in prenatal diagnosis**

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Objectives: We explore the possibilities for the application of the Affymetrix 250k SNP array platform in prenatal diagnosis.

Patients/methods: 250k Nspl SNP array analyses were carried out on DNA from 16 fetuses (after TOP; n=11 or IUFD; n=5) and 3 newborns, all prenatally karyotyped because of ultrasound anomalies and issued as normal (n=18) or as carrier of a *de novo* translocation (n=1). CNVs (gains>200kb and losses>150kb) were categorized as either benign or (possibly) clinically significant.

Results: Aberrations were detected in 6 cases. Three were highly likely clinically relevant and cytogenetically not visible: a *de novo* 2.9 Mb loss in 17p13 (IUFD), a 5 Mb loss in 3q26.33q27.2 (TOP; *de novo* t(3;18)(q26.2;q21.3)) and a maternal UPD 16 (live born child with MCA). In a fourth fetus a CNV with (yet) unknown clinical significance was detected: a 340 kb gain in 17q12 (TOP; no parental analysis so far). Furthermore, in a case of IUFD with no fetal karyotyping possible and mother being carrier of a t(4;22)(q12;q11.1), a 56.5 Mb gain of 4p16.3q12 was found. In a newborn with MCA, a 22qter deletion detected postnatally was further characterized and shown to be 6.1 Mb in size.

Conclusion: In 6/19 cases, genome-wide SNP array analysis enabled the detection of imbalances that otherwise would have remained undetected. Its high resolution increases the reliability for detecting imbalances, but more knowledge is essential to improve the interpretation of CNVs. Moreover, criteria need to be established for the conscientious application of prenatal genome-wide array analysis.

**P05.04****Prenatal diagnostic of Anomaly Dandy-Walker.**

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Dandy-Walker malformation is characterized by agenesis or hypoplasia of the cerebellar vermis, cystic dilatation of the fourth ventricle and enlargement of the posterior fossa.

Between 2000 and 2008 the Astrakhan Medico-Genetic Consulting Centre performed prenatal ultrasound diagnosis for 58220 women. It was found that 10 fetuses had the Dandy-Walker malformation. 3 women were diagnosed before 22 weeks of pregnancy and 7 - after 22.

4 fetuses were male, 6 were female.

For 7 of the positive fetuses it was their mother's first pregnancies, 2 - second and 1 - fourth pregnancy.

The main characteristic used to determine a positive test was an enlargement of the cisterna magna greater than 10 millimeters, complete aplasia/hypoplasia of the cerebellar vermis.

5 (50%) of the positive cases had fetuses that had hydrocephalus.

4 (40%) had chromosomal abnormalities: trisomy 18 (2) and mosaic monosomy X (2). All of the present chromosomal abnormalities were accompanied by defects within the corresponding patient's central nervous system (CNS) (dysgenesis of the corpus callosum, hypoplastic brain hemispheres, polymicrogyria) and other organs (congenital heart defects, clubfoot and omphalocele). 3 of the cases were accompanied by porencephaly (2) and microcephaly (1), 1 of the cases had cleft lip and palate.

One of the positive cases had associated CNS and non-CNS-associated malformation, other have short rib-polydactyly syndrome type 2. All of these results were verified through autopsy.

Using this data to analyze the rate of Dandy-Walker syndrome, its was found to be 0,017 % or 1 in 5822 fetuses.

**P05.05****Prenatal diagnosis of a bladder extrophy, a developmental pathology or a syndromic association ?**

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rest, Romania, <sup>4</sup>Titu Maiorescu University, Bucharest, Romania.

Bladder extrophy is caused by incomplete closure of the inferior part of the anterior abdominal wall. It has an incidence of 1/10000-1/40000 births and is more common in males (2,3M/1F). Separation of pubic bones, low set umbilicus and abnormal genitalia are associated anomalies.

We present a case of bladder extrophy who was diagnosed prenatally. It was referred to our Prenatal Department at 32 weeks of gestation for a suspicion of ambiguous genitalia.

The child was born prematur, at 36weeks. Because of presence of some dysmorphic features, like low nasal bridge and micrognathia we have thought to perform further investigations to establish if in this case is just an development defect or bladder extrophy is a part of a syndrome. Those aspects are important for prognosis and management of the case and also for recurrence risk.

**P05.06****Contribution of three-dimensional computed tomography in prenatal diagnosis of lethal infantile cortical hyperostosis (Caffey disease)**

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Infantile cortical hyperostosis (Caffey disease) is characterized by acute inflammation of soft tissues and profound alterations of the shape and structure of the underlying bones, particularly the long bones, mandible, clavicles or ribs. These manifestations generally appear during the first month of life, though time of onset varies, and a severe lethal prenatal form has been reported. The diagnosis of lethal prenatal Caffey disease is generally made post-mortem since the combination of two-dimensional ultrasound and antenatal foetal x-rays is not sufficient to make the diagnosis. Here we describe the contribution of three-dimensional helical computed tomography (3D-HCT) in the diagnosis of lethal Caffey disease in a 30 WG pregnant woman, evaluated for shortened and bowed long bones. Periosteal thickening of the diaphyses and cortical irregularities were in favour of the lethal form of infantile cortical hyperostosis. This diagnosis was confirmed by post-mortem x-rays and histological examination after elective termination of pregnancy. The search for the 3040C→T COL1A1 mutation on DNA extracted from foetal blood cells was negative. This case report is the first description of 3D-HCT in lethal Caffey disease. It further emphasize the value of 3D-HCT as a complementary diagnostic tool in the prenatal diagnosis of osteochondrodysplasia. This technique provides 3D images of the abnormal findings more readily, which could aid in counselling and management of the pregnancy.

**P05.07****Cell cycle studies of human cytotrophoblast cells in missed abortion**

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Abnormal proliferation and differentiation of trophoblast can lead to functional insufficiency of placenta and developmental destruction of embryo. Meanwhile available data on proliferative capacity and cell cycle of cytotrophoblast (CTB) in normal and pathological pregnancies are inconsistent.

Proliferative capacities of CTB of chorion villi samples (CVS) from missed and artificial abortions (groups M and A respectively) at 5-8 weeks of gestations were studied. After 24, 48, 72 hours incubation with BrdU, semi-direct preparations and AO staining the metaphases with SCD and SCE were registered. Group M included CVS with normal karyotype (N=4) and with heteroploidy (N=4). Group A included CVS with normal karyotype (N=6).

In spite on conspicuous interindividual variations attributed to the differences in mitotic activity and gestation ages, obvious differences were found. The number of cells with accomplished two S-phases after

24 h was equal to  $3,1 \pm 3,09\%$  and they were detected only in the group A, whereas after 48 h they were represented in both A ( $36,1 \pm 16,01\%$ ) and in M groups ( $10,5 \pm 4,20\%$ ) ( $P < 0,01$ ). There was no significant differences in the number of such cells ( $55,2 \pm 9,09\%$  and  $48,2 \pm 5,21\%$  respectively,  $P > 0,05$ ) after 72 h incubation. No 3<sup>rd</sup> generations cells have been registered in both groups.

These results are consistent with some previous data which postulate the duration of one complete cell cycle of CTB equal to approximately 36 h. However proliferative capacity *in vivo* of CTB cells from missed abortion is reduced if compared to this one of progressive normal pregnancy.

#### P05.08

#### Relationship of circulating cell-free DNA levels to cell-free fetal DNA levels, clinical characteristics and laboratory parameters in preeclampsia

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Objective: Elevated amounts of circulating DNA in maternal plasma have been detected in pregnancies complicated by preeclampsia. We attempted to confirm this and simultaneously examined whether increased circulating cell-free DNA levels are related to the clinical characteristics and laboratory parameters of preeclamptic patients.

Study design: Circulating DNA was measured by real-time PCR in plasma samples obtained from 67 women with preeclampsia and 70 normotensive pregnant women. Standard laboratory parameters and C-reactive protein levels were determined by an autoanalyzer. Plasma von Willebrand factor antigen levels were quantified by ELISA, while plasma fibronectin concentration by nephelometry. Plasma malondialdehyde levels were measured by the thiobarbituric acid-based colorimetric assay.

Results: We confirmed that circulating total free and fetal DNA levels are significantly elevated in pregnancies complicated by preeclampsia (median: 11.395 vs. 32.460 and 0.001 vs. 0.086 pg/ul;  $P < .001$ ). The quantity of total plasma-free DNA did not correlate with most of the laboratory parameters, except for serum aspartate aminotransferase and alanine aminotransferase activities (correlation coefficient: 0.31;  $P=0.012$  and 0.46;  $P<.001$ ). There was no correlation with clinical characteristics, including body mass index.

Conclusion: The releases of both free fetal and maternal DNA were found to be affected in preeclampsia. The quantity of markers of inflammation, endothelial activation/injury and oxidative stress did not show any correlation with cf DNA levels, and neither did cff DNA levels. Hepatocellular necrosis seems to be responsible - at least partly - for increased circulating total DNA levels in preeclampsia, as suggested by the significant correlation with liver enzyme activities

#### P05.09

#### Fetal sex determination by capillary electrophoresis from maternal plasma in pregnant women

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INTRODUCTION: Male specific fetal nucleic acid sequences from Y chromosome in pregnant woman maternal plasma DNA is quite easy to distinguish even if contribution of fetal DNA is very low. Most approaches that are reliably able to determine fetal sex are based on Real-Time PCR. Most labs dealing with cell free fetal DNA analyses have this method already routinely established.

Main objective of our study was optimization of noninvasive fetal sex detection from maternal plasma in pregnant women using capillary electrophoresis and prepare this procedure for quantitative analyses in our new project „Fragment and quantitative profiling of cell free fetal nucleic acids from maternal plasma in pathological and physiological pregnancies“ which is supported by IGA MZ CR No. NS/9624-3.

METHODS: Male/female artificial DNA mixtures and together 475 DNA samples isolated from maternal plasma in different weeks of pregnancy ranging from 4th w.g. to 37th w.g. were used for DNA analyses. Y chromosomal sequences in AMELY and TSPY were tested by refined quantitative fluorescent PCR using capillary electrophoresis.

RESULTS: The method is able to distinguish 1 % of male genome from male/female artificial mixtures. Investigation and assessment in cell

free fetal DNA samples achieved 4.05% of false positivity and 7.15% of false negativity in Y sequence detection.

CONCLUSION: Established method allows fetal sex detection with high sensitivity and specificity. The method has got also a potential for quantitative purposes.

#### P05.10

#### “False” positive cases in congenital adrenal hyperplasia (CAH) neonatal screening: usefulness of a CYP21A2 genotyping

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Aim: To evaluate the usefulness of a second-tier DNA analysis in CAH neonatal screening positive cases without clinical signs.

Patients And Methods: 58 patients (20f, 38m) with three positive 17OH-progesterone (17OHP) determinations, but no neonatal CAH signs after evaluation by paediatric endocrinologists, who indicated the DNA analysis. Serial determinations of 17OHP and clinical follow-up (>1y). Study of the common mutations (PCR-ASO) and R426H (7% virilizing forms in Spain) together with genomic DNA analysis for hybrid deletions/large gene conversions to detect hemizygosity, semiquantitative primer-extension for gene duplications carrying Q318X, microsatellite typing to detect unknown homozygosity for rare alleles (complementary sequencing).

Results: CYP21A2 segregated mutant alleles were detected in 13 patients: 3 compound heterozygous with I172N and null alleles (3 boys), 8 compound heterozygous with severe mutations and V281L, and 2 with two mild alleles. Sustained 17OHP levels confirmed CAH-21OHD diagnosis of these “neonatal cryptic forms”. In 43, mutations were discarded in both alleles and a carrier situation was detected in 4 (655G, 655G+Q318X,V281L, P453S). The normal variant Q318X in gene duplicated alleles was detected in 2 additional samples. Absence of clinical signs and normalization of 17OHP levels, in these remaining 45 cases, discarded the deficiency.

Conclusion: CYP21A2 genotyping proved useful as a second-tier analysis. The deficiency was discarded in all the negative patients and carriers. The genotypes in those CYP21A2 positive patients allowed to discard a severe deficiency and were compatible with mild forms in boys and girls (compound heterozygous with V281L) or virilizing forms in boys (compound heterozygous with I172N).

#### P05.11

#### Prenatal diagnosis of Dandy Walker malformation: case report

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A 35-year-old pregnant Caucasian female was referred at 20 weeks of gestation for a routine prenatal ultrasound. Fetal monitoring was made by ultrasound scans for fetal growth, congenital malformations, and amniotic fluid volume. Information about family medical history was collected too. Amniotic fluid samples were taken to perform prenatal cytogenetic diagnosis because sometimes Dandy Walker malformation is associated with chromosome aberrations. The woman was tested for TORCH. Results: Ultrasound examination revealed a singleton pregnancy with cerebellar malformation and associated anomalies: ventriculomegaly, meningocele and hydrocephaly. Karyotype indicated a normal cytogenetic female: 46, xx. No evidence of an infectious origin was found. The pregnancy was terminated at 25 weeks of gestation. Autopsy findings confirmed the ultrasound diagnosis. Conclusions: a sporadic case with Dandy Walker malformation was described; prenatal ultrasound disclosed a positive result useful for pregnancy management and counseling for abortion.

#### P05.12

#### Importance of Routine Ultrasonography in Detecting Fetal Karyotype Abnormalities in Low Risk Pregnancies

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First- and second-trimester ultrasonographic (USG) findings of fetal chromosome abnormalities include structural abnormalities and/or sonographic markers of fetal aneuploidy. Sonographic markers of fetal aneuploidy may be seen in normal fetuses and are often transient. We aimed to evaluate the importance of USG findings, in estimating cytogenetic abnormality risks in low aneuploidy risk pregnancies. We reviewed a number of most commonly accepted markers and structural abnormalities on pregnant women with low risk who underwent invasive prenatal diagnostic tests for USG abnormality in the period from January 2002 to December 2008. Prenatal genetic diagnosis of 68 cordocenteses, 42 chorionic villus samples (CVS) and 1688 amniocenteses (AS) were performed. In 179 of all cases (9.95%), cytogenetic analysis was recommended because of USG abnormality. 82 patients had structural abnormalities and 97 had sonographic markers. We detected 10 aneuploidies in fetuses with structural abnormalities and 3 aneuploidies in fetuses with sonographic markers, as expected. We concluded that, although the presence or absence of sonographic markers can substantially modify the risk of fetal aneuploidy, structural abnormalities inevitably have high risk for aneuploidies.

#### P05.13

#### Prenatal Detection of Pericentric Inversion of Chromosome 9 in 5358 Referrals at a Reference Genetic Center

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Pericentric inversion of chromosome 9 is a structural chromosomal variant which usually has no phenotypic effect and occurs 1.98 % in human population. In this study, we report on the incidence of pericentric inversions of chromosome 9, the indications of those results, and discuss the data in the light of the literature. We have reviewed the results of a total of 5358 pregnant women who underwent invasive prenatal procedures between January 1998 and December 2008. Inversion of chromosome 9 was detected in 60 of them (1.1%). The most common indications were advanced maternal age (AMA) [n: 27, (45%)], high risk result on triple test (HRRTT) [n: 14, (24%)], anomalies in USG examination [n: 10, (16%)], increased nuchal translucency [n: 4, (7%)], and other reasons in 5 (8%)[n: 5, (8%)]. In conclusion the incidence of prenatally detected pericentric inversion of chromosome 9 is compatible with the incidence of general population.

#### P05.14

#### Leptin gene (TTTC)<sub>n</sub> microsatellite polymorphism in pre-eclampsia and HELLP syndrome

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Genetic variation on the leptin gene (LEP) influences susceptibility to obesity. A study showed correlation between the length of the microsatellite on the LEP gene and pre-eclampsia. We decided to compare the tetranucleotide repeat (TTTC)<sub>n</sub> polymorphism in the 3'-flanking region in the leptin gene on DNA samples of patients with pre-eclampsia and HELLP syndrome. Blood samples were collected from normal pregnant (n=71), pre-eclamptic (n=64) and HELLP (n=69) syndrome patients. Fluorescent PCR and DNA fragment analyses was performed from isolated DNA. The electrophoretograms were evaluated and patients were assigned to two groups, class I low (<160 basepair) or class II high (≥160 basepair). We observed a higher frequency of class II alleles in the PE and HELLP syndrome patients, which was only shown to be significant in the case of HELLP syndrome (p=0.025) patients. The Pearson Chi-square test did not show significant differences in the genotype distribution. We observed a high frequency of II/II genotype in the PE (43.8%) and HELLP (56.5%) syndrome patients relative to the healthy controls (38%). Our results highlight the necessity of LEP (TTTC)<sub>n</sub> polymorphism studies.

#### P05.15

#### Leptin receptor (LEPR) SNP polymorphisms in HELLP syndrome patients determined by quantitative real-time PCR and melting curve analysis

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**Background:** Polymorphism of the leptin gene receptor (LEPR) may modulate the effect of elevated serum leptin levels in pre-eclampsia. The aim of this study was to determine four LEPR SNPs in HELLP syndrome patients.

**Methods:** DNA was isolated from 83 normal healthy pregnant controls and 75 HELLP syndrome patients' blood samples. Four SNPs LEPR G+5193A (K109), LEPR G+27265A (Q223R), LEPR G+44704C (K656N) and LEPR A+71001G (S1008) were determined by quantitative real-time PCR and melting curve analysis.

**Results:** The allele and genotype distribution of the four LEPR SNP was not significantly different in HELLP syndrome patients compared to the healthy pregnant controls. However, there was a difference in the occurrence of the LEPR G+5193A (K109) GA genotype, which was twice more frequent in HELLP syndrome patients(40% vs. 24%) (p=0.089). **Conclusion:** We did not find a difference in the allele and genotype distribution of the four studied LEPR SNPs between HELLP syndrome patients and the healthy controls. The quantitative real-time PCR combined with melting curve analysis is a fast and reliable method for the determination of LEPR SNPs

#### P05.16

#### Limb Body Wall Complex- A Case presentation and review of literature

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**Background.** Limb Body Wall Complex (LBWC) is a combination of development abnormalities involving the neural tube, body wall and the limbs. There are few cases in literature, and our case is only the 2nd presented from Romania.

**Case presentation.** The patient was a 31 year-old women G1P0A0 with a 33 week pregnancy which had no prenatal care. The ultrasound scan described several abnormalities, including: large abdominal wall defect, with difficult to identify pelvic organs and ambiguous genitalia; enlarged stomach with suspicion of intestinal atresia; scoliosis and spina bifida occulta with bilateral ventriculomegaly; one inferior limb absent; short umbilical cord with single artery. After therapeutic termination of pregnancy, the abnormalities were confirmed and polycystic liver and kidneys were also mentioned. Also bilateral cardiac ventriculomegaly, left superior pulmonary lobe hemorrhage, imperforated anus and pancreas agenesis were identified. No abnormalities were found at chromosomal examination - 46,XY.

**Conclusion.** The case presented here is a placento-caudal phenotype of a LBWC syndrome, which had as a special element the polycystic kidney and hepatic disease. The description of plurimalformative features in our case is important to better understanding of pathogenesis of disease.

#### P05.17

#### Detection of Maternal Cell Contamination (MCC) using STR/microsatellite markers on a Capillary Electrophoresis System

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Typically prenatal genetic testing requires fetal gDNA that is isolated from amniotic fluid (AF) and chorionic villus samples (CVS). AF and CVS samples are extremely valuable samples that are collected via invasive surgical procedures, and as a result are subject to maternal cell contamination (MCC). Due to the sensitivity of PCR, MCC can potentially contribute a significant source of error in prenatal testing with misinterpretation of a prenatal test thought to be possible at levels of MCC as low as 1-2%. Commercially available forensic human identification kits that employ fluorescent multiplex PCR amplification of polymorphic microsatellite repeats are suitable for the MCC detection. To achieve several informative markers when analyzing samples for MCC, we utilized the AmpFLSTR® Minifiler™ PCR Amplification Kit, which interrogates 8 microsatellite loci across four fluorescent dyes. In order to evaluate a new capillary electrophoresis (CE) instrument, synthetic MCC sensitivity controls (10%, 5%, and 1%) as well AF samples were analyzed. Semi-quantitative calculations of the level of

MCC contamination were performed by comparing the peak area of the informative maternal allele to the unique fetal allele; the new CE instrument provided adequate sensitivity for detecting a synthetic MCC control at a level of 1% or greater. Further, we discuss common pitfalls to the analysis of MCC results such as broad peak errors, allelic drop out (ADO), preferential allelic amplification, and the influence of stutter peaks.

#### P05.18

#### Expression Profiling of Placental microRNAs in Preeclamptic Maternal Plasma

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Preeclampsia is a potentially dangerous disorder specific to the second half of pregnancy, affecting about 2.5-3% of women. It is the most frequent pregnancy associated disorder and still a leading cause of maternal and perinatal mortality and morbidity and preterm birth. Preeclampsia starts with placental dysfunction in the first trimester. Despite extensive research on preeclampsia during the last decades, the underlying pathogenetic mechanisms remain unclear.

Identification of placental products rather than cells in the maternal circulation has taken a drastic turn for the better by the observation that cell-free DNA and RNA originating from the placenta circulates in a protected form in the maternal blood and can be obtained easily from the maternal plasma. Recent studies on microRNAs (miRNAs) offer possibilities for developing another class of molecular markers. miRNAs are short (19-25 nucleotides), single-stranded, and nonprotein-coding RNAs that regulate gene expression by binding to the 3' untranslated region of the target mRNAs.

In this study, it is aimed that detection of miRNAs which have the different expression profile in maternal plasma in preeclamptic pregnant compare to healthy pregnant. In the content of study, miRNA extraction from maternal plasma of 20 pregnant with preeclampsia and 20 non-preeclampsia healthy pregnant, detection of expression differences by the use of microarray and expression analysis of detected miRNAs by quantitative real time pcr. The comparison of the collection, isolation and concentration approaches as well as detection can be observed in detail on our poster presentation.

#### P05.19

#### Study of the natriuretic peptide precursor gene (TTTC) repeats in essential hypertension and preeclampsia

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**Background:** Lately a novel variable tandem repeat polymorphism (TTTC) in the 5'-flanking region of the natriuretic peptide precursor B gene (NPPB) was discovered, which was associated with essential hypertension. It has not been studied in preeclampsia (PE). PE is a serious complication of pregnancy with hypertension and proteinuria, developing in the second half of the pregnancy. We decided to determine the tandem repeats of the NPPB gene in preeclampsia.

**Method:** DNA was isolated using High Pure PCR Template Preparation kit (Roche, Germany) from blood samples of 28 healthy pregnant, 17 pregnant with essential hypertension and 26 preeclamptic patients. We used fluorescent PCR and DNA fragment analysis for the detection of the NPPB gene's tandem repeat units on ABI 3130.

**Results:** We found that the distribution of the TTTC repeat number is giving mainly two groups, one at 11 and the other at 16. We found low frequency of the high number of repeats (n=16) in the control healthy group (21.4%), while it was higher in essential hypertension (32.3%) and preeclamptic patients (32.6%). **Conclusion:** We found higher frequency of the high TTTC repeat (n=16) alleles of the NPPB gene in essential hypertension and preeclampsia. We continue our investigation to find out the role of this polymorphism in the development of hypertension.

#### P05.20

#### Identification of *UGT1A1* (TA)<sub>5</sub> and (TA)<sub>8</sub> alleles in Slovenian newborns with pathological unconjugated hyperbilirubinemia

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Unconjugated hyperbilirubinemia is a common finding during the neonatal period and can sometimes lead to severe consequences such as bilirubin encephalopathy. Increased number of TA repeats in the promoter region of the bilirubin UDP-glucuronosyltransferase gene (*UGT1A1*) results in decreased bilirubin conjugation and is the principal cause of Gilbert's syndrome in Caucasians.

The aim of the present study was to investigate whether *UGT1A1*(TA)<sub>n</sub> polymorphism was related to pathological hyperbilirubinemia of unexplained aetiology in Slovenian newborns.

Single-strand conformation polymorphism analysis was used to genotype 147 consecutive newborns with unexplained jaundice (total serum bilirubin over 220 µmol/L) admitted to the Neonatal unit of University Medical Centre Ljubljana.

The frequencies of genotypes were as follows: (TA)<sub>6/6</sub> (42.9%), (TA)<sub>6/7</sub> (37.4%), (TA)<sub>7/7</sub> (16.3 %), (TA)<sub>5/6</sub> (2.0%), (TA)<sub>5/7</sub> (0.7%) and (TA)<sub>6/8</sub> (0.7%). The frequency of (TA)<sub>7/7</sub> genotype, which is characteristic for Gilbert's syndrome did not differ from that of the healthy Slovenian population ( $p>0.05$ ). However, in neonatal hyperbilirubinemia group males had higher frequency of (TA)<sub>7</sub> allele than females ( $p=0.045$ ). It is interesting that there were 69.4 % males and 30.6% females in our hyperbilirubinemia group.

In conclusion, frequency of *UGT1A1*(TA)<sub>n</sub> polymorphism genotypes was determined for the first time in Slovenian newborns with pathological hyperbilirubinemia. The extremely rare (TA)<sub>5</sub> and (TA)<sub>8</sub> alleles in Caucasians were found also in Slovenians. Our results suggest that male newborns with (TA)<sub>7</sub> allele have an increased risk to develop pathological neonatal hyperbilirubinemia.

#### P05.21

#### Recurrent progressive non-immune hydrops foetalis: better think of mucopolysaccharidoses type VII/ beta-glucuronidase deficiency/ *GUSB* gene defect

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Mucopolysaccharidoses type VII/ beta-glucuronidase is an autosomal recessive lysosomal storage disease. The phenotype is highly variable, ranging from severe lethal non-immune hydrops foetalis to mild forms with survival into adulthood. We describe a non-consanguineous couple, first presented with non-immune hydrops foetalis, ascites and clubfeet in the fourth pregnancy at 20 weeks of gestation. They had one early miscarriage and two healthy children. Prenatal diagnosis showed (familial) 45,XX, der(14;15)(q10;q10) karyotype with *de novo* RAI1 locus duplication MLPA detected. Uniparental disomy 14 and 15 were excluded. The pregnancy was terminated at 26 weeks of gestation. Lysosomal enzyme investigations in amniotic fluid were not conclusive and 5% recurrence risk for hydrops foetalis was counselled. In the fifth pregnancy prenatal diagnosis revealed a 46,XY karyotype without RAI1 duplication. However, ultrasound investigation showed again a progressive hydrops foetalis. Cultured amniocytes

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from the fourth pregnancy combined with amniotic fluid investigation of the fifth pregnancy using glycosaminoglycan electrophoresis and lysosomal enzyme activity measurements confirmed beta-glucuronidase deficiency in both. Cord blood investigation revealed prominently vacuolated monocytes, with basophilic inclusions in lymphocytes (Gasser cells) and metachromatic granules (Alder-Reilly bodies) in granulocytes. Electron microscopic investigations showed vacuolated Hofbauer cells in the placenta and foamy macrophages in spleen, liver, and other organs. Greatly reduced activity of beta-glucuronidase in cultured skin fibroblasts reconfirmed the diagnosis of MPS type VII and *GUSB* DNA analysis is still pending. We can conclude that recurrent massive non-immune hydrops foetalis is likely autosomal recessive with 25% recurrence risk in which lysosomal storage disorders need to be investigated.

#### P05.22

##### Achivements in the NIPD of monogenic disorders: a prenatal diagnosis unit experience

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Non-invasive prenatal diagnosis (NIPD) using fetal DNA in maternal plasma has proven its potential for the study of the fetus. Many diagnostic units are currently offering non-invasive fetal sex assessment for X-linked disorders and Rh determination in Rh(-) pregnant women.

As for the monogenic disorders, since the fetal DNA circulates within a high background of maternal DNA, NIPD is mainly based on the study of paternal alleles, absent in the maternal genome. This limits this approach to pregnancies in which the father is carrier of an autosomal dominant mutation or those in which both parents are carriers for different autosomal recessive mutations.

The clinical incorporation of NIPD for monogenic disorders must fulfil the same needs of the conventional molecular prenatal diagnosis.

We have studied 14 paternal mutations associated to different diseases: X-linked retinitis pigmentosa, congenital Leber amaurosis, cystic fibrosis, propionic acidemia and Huntington disease.

The diagnosis was performed by the use of different analytical methods currently used in the clinical practice.

The fetal condition for the paternal mutation was correctly diagnosed in 13 out of the 14 cases. The remaining case could not be diagnosed due to the length of the mutant allele.

NIPD of monogenic diseases could be the forthcoming study to be offered in clinical practice. The study of monogenic disorders in maternal plasma must be individualized and directed. As a Prenatal Diagnosis Unit we are aware of the importance of having different diagnostic tools available to get to a reliable diagnosis for each individual case.

#### P05.23

##### A case of partial trisomy 3q in a fetus

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A married couple underwent cytogenetic testing following the detection of abnormalities in a fetus 25 weeks. The father was 22 years old, the mother was 23 years old, and this was their 3<sup>rd</sup> pregnancy. Fetal ultrasound had revealed multiple congenital abnormalities including congenital heart disease, common arterial trunk, a 4mm defect of interventricular septum, and Dandy-Walker syndrome. The mother's karyotype was 46,xx, inv (3) (p2.5 q2.1). Cordocentesis allowed karyotyping of the fetus and revealed: 46,xx,der(3) (3qter-cen 3p2.5::3q2.1-3qter), resulting in partial trisomy of the long arm of the 3<sup>rd</sup> chromosome as a result of recombination inv(3) by the mother. The pregnancy was subsequently aborted. Fetal pathology revealed: body mass 570g, length 21cm. The sex was indeterminate. There was acrocephaly, high forehead, and hypertelorism. The nose is small and flat with broad nostrils, and the bridge was sunken. The ears were low.

The fetus also had contracture of knee and talocrucal joints. The lower limbs were adducted to the trunk, and there was lower limb syndactyly. The upper extremities are without pathology. There was agenesis of the urinary bladder, the ureters flowing into a broadened rectum. No internal sexual organs were detected.

#### P05.24

##### Overview of Prenatal Diagnosis (PND) in our Country (Iran)

**M. H. Kariminejad, Azadeh Moshtagh, Farnaz Azimi, Nasrin**

*NabaviNia, Mahnaz Pirveissi, Narges Miraftabi, Roxana Kariminejad; Kariminejad & Najmabadi Pathology & Genetics Center, Tehran, Islamic Republic of Iran.*

Prenatal diagnosis became a generally accepted tool for prevention of genetics disorders since the 1980s. Simultaneously Iranian geneticists and clinicians of related fields attempted to establish this procedure in our country.

It was a great challenge, it took a long time to be overcome the problems, including legal approval initially granted for hemoglobinopathies at 1996 and thereafter for other genetic disorders including chromosomal abnormalities, metabolic diseases MPS, lipid lysosomal storage diseases: Single aminoacids, muscular dystrophies, myopathies (SMA), triple nucleotide repeats, Skin lesions and mitochondrial diseases.

Hereby the results, and the practical problems encountered in eleven thousand chromosomal study of amniotic fluid samples are presented.

The pregnant ladies are classified according to clinical indications as follows: Maternal age over 30 years, history of offspring with chromosomal aberration, the similar group with advanced maternal age, balanced chromosomal aberration in either parents, loss of offspring pre or postnatal death, high sick of chromosomal abnormalities according to triple, quadruple, and 1<sup>st</sup> trimester fetal screening tests.

#### P05.25

##### Prenatal Down's syndrome screening in St.Petersburg in 2008

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There is a growing interest in the shift of prenatal screening to the first trimester. Such screening relies on the maternal age, free  $\beta$ -hCG, PAPP-A plus NT. The second trimester screening in Saint-Petersburg is performed for all pregnant women before 35, the first trimester screening - for all at the age 35 and more. Since 2004 combined screening was initiated in Saint-Petersburg. Down syndrome (DS) detection rate was 94.5% (52/55) (cut off 1/250, FPR 5.8%). Altogether 42111 (82.5%) pregnancies were tested in 15-17 weeks. Detection rate in 2-d trimester was 75% (24 of 32) (1/360, FPR 7.0%). 2705 of pregnant women of 35 age and more (36%) were tested in first trimester. Detection rate in 1-t trimester was 100% (17 of 17) (1/250, FPR 16.0%). Many women from them participated in the both screenings. Since 2008, February the simple calculation formula has been suggested for DS risk estimation in the women subjected to both screening tests. According to this formula the final risk is equal to the risk of the 1st trimester multiplied by the risk of the second one and divided by age risk. In that case the number of FPR reduces almost twice. But application of two steps screening is economically unjustified. Second screening could be recommended only to the women with risk about 0.05 and 0.4% after the 1<sup>st</sup> trimester screening. This group contributed only 17% of all pregnancies in 2008. More sophisticated decision concerning application of contingent screening could be taken after some economical considerations.

#### P05.26

##### Prenatal diagnosis of Mucopolysaccharidoses in Egypt: counseling aspects, sampling techniques and biochemical diagnosis

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Objective: prenatal diagnosis of mucopolysaccharidoses in pregnant females with previously affected child

Subjects: The present study included 35 pregnant females with previously affected MPS child or more. 10 type I (Hurler), 9 type II (Hunter), 5 type IIIb (Sanfilippo), 3 type IVa (Morquio) and 8 type VI (Maroteaux-Lamy)

Consanguineous marriage was present in 30 (85.7%) couples. 15 families have no normal children and 20 families have normal children. However families with no normal males are 27.

All the pregnant females were subjected to history taking, pedigree construction, clinical examination and ultrasound scan. Proper counseling was done and patients were scheduled for prenatal diagnosis. 4 cases did not come in scheduled time, 3 of them were type II and one was type IIIb.

5 females (3 type I and 2 type IIIb) came in two successive pregnancies for prenatal diagnosis to prenatal diagnosis was done in 2 pregnancies of them as anembryonic sacs were diagnosed. Another female (MPS VI) came in three successive pregnancies. However prenatal diagnosis was not performed in two of them as the first was vesicular mole and the second was anembryonic sac, both ended by evacuation

#### P05.27

#### Molecular Genetic Tests and Referral Reasons in Prenatal Diagnosis at a Tertiary Referral Hospital

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Ege University Faculty of Medicine, Izmir, Turkey.

Prenatal diagnosis is helpful for planning the problems that may occur in the newborn period and deciding whether to continue the pregnancy. Single gene mutations as the cause of single gene disorders can be detected by molecular analysis in the prenatal period. The prevalence of all single gene disorders at birth is about 10 per thousand. It is possible to apply the molecular genetic analyses in these patients for prenatal diagnosis and genetic counseling. Here, we present the most common requested single gene disorders and molecular genetic tests in our center. The molecular test results of all prenatal referrals between 2006-2009 at a tertiary medical center in western part of Turkey were evaluated retrospectively. Total 121 prenatal samples were included in the study. Ninety-four of 121 prenatal tests were performed for beta-thalassemia (77.7%) which was followed by cystic fibrosis in 16 (13.2%), 10 in spinal muscular atrophy (8.3%), 1 in Osteogenesis imperfecta (0.8%). Because beta-thalassemia is the most common single gene disorder and one of the most important health problems in Turkey, particularly, prenatal diagnosis of this disease has become a widely available test.

#### P05.28

#### Prenatal diagnostics in Bulgaria - current experience and future trends

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Invasive prenatal diagnostics was introduced into medical practice in the early 70-ies. Since then it is recognized as a useful tool for prevention of severe human diseases. Prenatal invasive diagnostics for monogenic and chromosomal diseases in Bulgaria started in 1980. Since 1996 maternal serum screening for Down syndrome and NTDs is routinely offered. Now National genetic laboratory has well developed services providing laboratory prenatal diagnostics of monogenic and chromosomal diseases in high risk families.

For a 28 years period 400 families with severe monogenic diseases as CF, DMD/BMD, SMA, beta-Thalassemia, PKU, CMT1A and other rare metabolic diseases underwent DNA prenatal diagnostics. In 55 families with lysosomal storage diseases prenatal diagnostics was performed by enzymatic assays. Among all families studied 91 fetuses were found to be affected. In some families new disease causing mutations were found.

During the last 5 years rapid growth of prenatal diagnoses for chromosomal diseases is evident and more than 1000 invasive procedures for this indication are performed yearly. As an alternative to cytogenetic analysis Q-PCR for common aneuploidies (trisomy 21, 18 and 13) was introduced in 1999 and since 2002 is used routinely in our practice. For 2008 among 490 amniocenteses performed because of risk over 1:250 after maternal serum screening 15 fetuses (3,1%) with trisomy 21 were found.

Since the capacity for cytogenetic analysis is limited and targeted Q-PCR detects only common aneuploidies the introduction of new technologies as arrayCGH seems to be a reliable tool for effective prenatal diagnosis of chromosomal diseases.

#### P05.29

#### Evaluation of the fetus with cheilognathopatatoschisis

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Background: Cheilognathopatatoschisis or complex cleft (involves cleft of the lip, upper jaw, and hard and soft palates) is a severe birth defect. It occurs isolated or associated with other medical conditions.

Aim: to determine whether the cheilognathopatatoschisis is syndromic or non-syndromic.

Patients and Methods: A 29-year-old Caucasian female, pregnant for the first time, was referred at 17 weeks' gestation for a routine prenatal ultrasound. The couple had normal general health and was not consanguineous. There was no family history of cheilognathopatatoschisis. Routine ultrasonography at 17 weeks of pregnancy, triple test (AFP, uE3, hCG), selective ultrasonography for detection of fetal abnormalities, and amniocentesis were performed.

Results: Ultrasound examination revealed a single fetus with an orofacial cleft and no other developmental abnormalities. Triple test was not sensitive to the presence of trisomy 13 but chromosome analysis was recommended because orofacial cleft as a sonographic marker suggested the possibility of a chromosomal anomaly. FISH and QF-PCR detected no aneuploidy in chromosomes X, Y, 13, 18 and 21.

Conclusions: Cheilognathopatatoschisis was diagnosed by performing 3D US in the second trimester of pregnancy. Our results suggested a non-syndromic cleft. Prenatal evaluation was useful in management, prognosis and prevented the negative emotional effects of the parents.

#### P05.30

#### Angiotensin converting enzyme (ACE) polymorphism and its role in the neonatal respiratory disease development

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Angiotensin converting enzyme plays an essential role in two physiological systems, one leading to the production of angiotensin II and the other to the degradation of bradykinin. The wide distribution and multifunctional properties of these peptides suggest that ACE could be involved in various pathophysiological conditions, including neonatal respiratory distress-syndrome (RDS) and pneumonia. The ACE levels are under genetic control, and the insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene is used as a marker for a functional polymorphism.

We investigated the role of ACE I/D polymorphism on the incidence of RDS and pneumonia. We studied 161 patients with RDS (n=97), congenital pneumonia (CP) (n=64) and 286 healthy term neonates matched by sex from Ufa, Russian Federation, using two primers PCR for each DNA sample twice.

For all samples, genotype distribution was in Hardy-Weinberg equilibrium. II genotype frequency was decreased in all patients (19.88%, p=0.005), including RDS (20.62%, p=0.031) and CP (18.75%, p=0.038), compared with the healthy term neonates (32.87%). The RDS patients with D allele (53.09%, p=0.026, OR=1.2), but not the CP group (51.56%, p=0.12), had the slightly increased risk of disease (43.53% in healthy term neonates group).

These data suggest a potential role for angiotensin-converting enzyme polymorphism in the development of neonatal respiratory disease. The D allele may adversely influence the risk of the RDS development. However this allele can be associated with birth prematurity of the RDS patients. So, further study is needed to confirm the association.

#### P05.31

#### Respiratory distress syndrome in neonate and association of polymorphisms surfactant protein D gene

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The surfactant proteins play important roles in lung function, and genetic variants of these proteins have been linked with lung diseases, including respiratory distress syndrome (RDS). The aim of the study was the investigation of association between polymorphisms of surfactant protein D gene (SFTPD (Met11Thr, Ala160Thr) and the risk of

developing RDS in neonates from Bashkortostan Republic, Russia. The whole peripheral blood of 159 patients with respiratory disease and umbilical cord blood of 299 healthy term neonates was used for the isolation of genomic DNA. The patient group consisted of 3 subgroups (63 neonates with pneumonia, 40 babies with RDS and 53 infectious complicated RDS) The genomic DNA served for PCR in the genotype analysis.

The results demonstrated that SFTPD (Met11Thr, Ala160Thr) gene genotypes frequency distribution patterns not significantly differ between patients with RDS and healthy neonates ( $\chi^2=2.68$ , df=2,  $p=0.262$ ;  $\chi^2=1.31$ , df=2,  $p=0.518$ ).

The SFTPD Thr/Thr genotype is protective against the development complication in RDS (7.5% vs. 32.5%;  $\chi^2=7.9$ ,  $p=0.006$ ; OR=0.17, 95%CI 0.04-0.64). Whereas, children with RDS who has the Met/Thr genotype were susceptible to infectious complication (73.6% vs. 50%;  $\chi^2=4.5$ ,  $p=0.003$ ; OR=2.79, 95%CI 1.07-7.32).

We suppose that the Met11Thr polymorphism of SFTPD gene may play a significant role in the development of complicated RDS in neonates.

### P05.32

#### Prenatal diagnosis of a fetus with ring chromosome 21 characterized by molecular cytogenetic methods

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Ring chromosome 21 is a rare structural chromosomal abnormality resulting, most of the times, from breakage in both arms of the chromosome and subsequent fusion of the two ends. There is a wide spectrum of phenotypes in ring 21 carriers that seems to be associated with different breakpoints of 21p and q arms, but also with somatic loss of the ring. Here we report a case of a fetus that was diagnosed with mos46,XY,r(21)(p11.2q22)[34]/45,XY,-21[4]/46,XY[14] karyotype. Ultrasound examination at 21 weeks' gestation showed no abnormalities and amniocentesis opted due to advanced maternal age. Cytogenetic analysis was performed in the parents and showed the presence of the ring chromosome in 1 out of 100 metaphases in the father, indicating a possible familial transmission. Analysis by Fluorescent In Situ Hybridization (FISH) and comparative genomic hybridization (aCGH) with resolution of 144 kb showed no deletion or duplication. After genetic counseling, the parents decided to continue the pregnancy and postnatal examination just after birth found no congenital abnormalities.

### P05.33

#### Robinow syndrome revealed during pregnancy on limb anomalies

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Robinow syndrome is a rare disease characterised by the association of mesomelic limb shortening, facial and genital anomalies. It can be inherited in an autosomal dominant or recessive mode. Recessive forms are severe and the result of ROR2 mutations, whereas the aetiology of dominant forms remains unknown. We report a further case of Robinow syndrome diagnosed at birth, with interesting prenatal findings.

Parents were healthy and not consanguineous. The mother was 39-year-old. The first trimester ultrasound showed an increased nuchal translucency (2.7 mm). Karyotype on amniotic fluid was normal (46, XX). The second trimester ultrasound revealed short ulna and radii (5<sup>th</sup> centile). A spiral CT scan revealed delayed ossification and a mesoaxial polydactyly on both feet. A chondrodysplasia was suggested and pregnancy was followed since no definite diagnosis could be realised. The baby girl was born at 39WG with a normal weight and head circumference but a decreased length (47 cm, 25<sup>th</sup> centile). Characteristic facial features were noted comprising hypertelorism, short upturned nose and gum hypertrophy. Mesomelic limb shortening and bilateral polydactyly of the feet were observed. Skeletal X-rays confirmed the bilateral mesoaxial polydactyly, revealed bifid terminal phalanges of both thumbs and did not show any vertebral anomalies. Cardiac, trans-

fontanellar, renal ultrasonography and eye examination were normal. Robinow syndrome was then diagnosed.

ROR2 molecular analysis was normal. Array-CGH is pending.

Even though Robinow syndrome has, to our knowledge, never been diagnosed during pregnancy on ultrasound findings, it should be suggested on the association of short long bones and mesoaxial polydactyly.

### P05.34

#### Split hand-split foot malformation due to 10q24 duplication identified during pregnancy on array-CGH

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Ectrodactyly or split hand-split foot malformation (SHFM) is a rare condition that occurs in 1 in 8500-25000 newborns and accounts for around 15 % of all limb reduction defects. SHFM is clinically heterogeneous and can be either isolated, associated with other malformations or part of syndromic entities. This condition is usually inherited in an autosomal dominant manner and several loci have been identified. Among them, SHFM3 has been located on 10q24 and the naturally occurring *Dactylaplasia* mouse is the animal model for SHFM3 in humans. Recently, 0.5 Mb tandem genomic duplications at chromosome 10q24 involving at least the *DACTYLIN* gene have been found in SHFM3 patients. No point mutations in any of the genes residing within the duplicated region have been reported so far, and it is still not clear how this rearrangement leads to the SHFM3 phenotype. Indeed, complex alterations of gene regulation mechanisms that would impair limb morphogenesis are likely.

We report on the third pregnancy of healthy non consanguineous parents presenting normal hands and feet. At 23WG, the routine ultrasound scan identified a split hand-split foot malformation. Chromosomes on amniotic fluid were normal 46XX and array-CGH shown a *de novo* 400 kb 10q24.31q24.32 duplication, comprising 5 genes including *DACTYLIN*. Reassuring genetic counselling was performed since these forms of SHFM are isolated and non-syndromic. At birth, limb anomalies were confirmed and corrective surgery performed.

To our knowledge, this is the first prenatal report of 10q24 duplication in SHFM.

### P05.35

#### Antenatal population carrier screening for spinal muscular atrophy

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##### Background

SMA is one of the most common autosomal recessive diseases with a carrier frequency of approximately to 1 in 50. Due to the incidence and severity of SMA and the availability of a carrier-screening test, we embarked on a stepwise prenatal SMA carrier screening program in Taiwan.

##### Methods

A total of 70,048 pregnant women participated in this carrier-screening program from 2005 to 2008. In the first phase, we used DHPLC as the single tool for genetic testing. In the second phase, we combined the DHPLC and MLPA for the test. If a woman was found to be a SMA carrier, her husband was asked to take the genetic test. If they both were SMA carriers, prenatal genetic diagnosis was suggested.

##### Results

A total of 40,756 pregnant women received the single-tool screening; 1,726 (4.2%) were discovered to be at high risk of being SMA carriers. Of these 1,726 couples, 62 husbands were also at high risk of being SMA carriers. Five fetuses from these couples were affected SMA cases. A total of 29,292 pregnant women received the combined tests; the results showed 603 (2.1%) were SMA carriers; twenty husbands were SMA carriers. Six fetuses from this group were affected SMA cases. This screening program cost \$401,202 US dollars to avoid a SMA affected child being born.

##### Conclusion

This was the largest study of population SMA carrier screening. Antenatal population carrier screening for SMA is feasible, effective and reasonable.

#### P05.36

##### Urge of prenatal diagnosis in modernized civilization

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Industrial revolution and modernization of medical sciences has contributed largely to expand the population and extend human lifespan. Massive quantities of toxic wastes are dispensed in the atmosphere unchecked as a direct consequence of our dependence on fossil fuels and advanced technologies which are rendering adverse effects on the nature. The recent decade has seen an unprecedented rise in natural calamities due to drastic fluctuations in weather conditions. These factors yield cumulative stress on the population causing tremendous damage to their health and well being. Infertility among adults, disorders like diabetes, cancer, HIV - AIDS, faulty births of infants having congenital abnormality and/or mental disorders are increasing at an alarming rate reducing the productive efficiency and profit derivative of the society.

Hence it is the need of the hour to provide a strong and healthy start to the future generations so that they can cope up with the ever increasing hardships. This can be assured if the new born children are free of debilitating ailments acquired before or during parturition. Prenatal diagnosis is already a routine measure to screen and prevent birth of genetically impaired children in well informed societies. However, this practice is limited at large due to lack of awareness among developing nations and availability of skilled diagnostic services in cost-effective manner. Conventional cytogenetics is one field of study that can prove to be an effective mode of prenatal diagnosis which should be given impetus by the organizations monitoring global health to bring immediate redress.

#### P05.37

##### Exposure to Fluoroquinolones during Pregnancy: An Analysis of Birth Outcomes from a Romanian Pregnancy Cohort Study

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**Background:** The new fluoroquinolones have favorable pharmacokinetic properties and high affinity for immature cartilage and bone tissue.

**Objective:** to evaluate the teratogenic risk following gestational exposure to fluoroquinolones, numbers of fetal deaths, distress, and the development of the toddler's skeleton at the age of twelve month.

**Methods:** A longitudinal, randomized, 6 year comparative study, from November 2002 to December 2008, was conducted in two primary medical care centers. We followed up 110 women exposed to anti-infective drugs during gestation, divided in 2 groups: a *study group* of 58 (52.72%) who took ciprofloxacin, norfloxacin, or ofloxacin for different infections and a *control group* of 42 (38.18%) exposed to macrolides.

**Results:** 23 women (25.21%) took fluoroquinolones in the first trimester of pregnancy, and there was a higher rate of therapeutic abortions among the study group (8 of 58 versus 3 of 42 - for the control group). Rates of major congenital malformations didn't differ between the two groups (relative risk, 0.88; 95% confidence interval, 0.27 to 3.29). The major malformations from the quinolone group were: hip dysplasia, hydronephrosis and ventricular septal defects, occurred after fluoroquinolone exposure during organogenesis. The relationship between fluoroquinolone exposure and arthropathy focused on the newborn's cartilage status (*craniotabes*) and on long-term musculoskeletal dysfunctions until at the age of twelve month (*diastasis recti* and *genu valgum*).

**Conclusions:** Gestational fluoroquinolone exposure is risky in the first trimester of pregnancy. Ultrasonography is needed to monitor the fetal development, followed by long-term musculoskeletal survey.

#### P05.38

##### Recurrent trisomy 21: Postzygotic formation of i(21q) from both maternal chromosomes 21

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Structural rearrangements involving chromosome 21 are responsible for 5% of trisomy 21 cases. The majority of rea(21q;21q) are, in fact, isochromosomes - i(21q) and only a small percentage are true Robertsonian translocations. The recurrence of de novo structural and numerical/structural chromosomal rearrangements might have three major reasons: a) recurrence by chance; b) gonadal mosaicism and c) low-level somatic-gonadal mosaicism in one parent.

We present a family with recurrent trisomy 21 in three consecutive pregnancies with maternally derived rea(21q;21q) in all cases. Extensive G-banded karyotyping and FISH analysis of the maternal peripheral blood revealed trisomy 21 mosaicism (1.5% cells were trisomic for chromosome 21). Gonadal mosaicism for trisomy 21 in a significant proportion of mother's ovarian cells provides a plausible explanation for her obstetric history.

Molecular studies using highly polymorphic short tandem repeat (STR) markers localised on chromosome 21 permitted constructions of haplotypes in three patient foetuses from three consecutive pregnancies. It revealed trisomy 21 was due to the isochromosomes, thus strongly suggesting their postzygotic origin. Most surprisingly, molecular analysis also demonstrated that isochromosomes in two cases originated from different maternal chromosomes 21.

Moreover, pregnancy outcome was also influenced by partners' consanguinity. After two unsuccessful attempts of IVF-PGS they got spontaneously pregnant. The child with normal chromosome 21 suffered from an autosomal recessive disorder (LCHAD / long chain 3-hydroxyacyl-CoA dehydrogenase deficiency) as a direct consequence of consanguinity.

To our knowledge, we demonstrate the first case of mosaics with two different maternal i(21q) derived from both maternal chromosomes 21.

#### P05.39

##### The analysis of mitochondrial DNA polymorphism in monozygotic twins

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Since the elucidation of the mitochondrial genome sequence in 1981, nearly 200 pathogenic point mutations, deletions, insertions and rearrangements have been described and associated with a variety of mtDNA-related human diseases. Due to unique twin genetics, twins have been identified as an ideal subject for observations of complex diseases inheritance. Still, so far no wide range analysis of mtDNA in twins has been reported. In this study the analysis of mitochondrial DNA polymorphism in group of monozygotic twins was performed, using umbilical cord blood. Zygosity of twins was determined by the analyses of DNA fingerprinting and mini- and microsatellite polymorphism (hybridization, PCR). The SSCP and HD analyses of D-loop mtDNA reveal a high polymorphism of this region. However, within the analyzed group of twins, no differences were found in either pair of monozygotic twins. T>C transition was detected in mtDNA sequence in one pair of twins. The resultant tract of 10 C preceded by 4 A seems to be related to heteroplasmy of mitochondrial genome.

#### P05.40

##### Outcome of fetuses with a sonographic finding of echogenic bowel.

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**Introduction:** Routine medical follow-up of pregnancies and development of ultrasonography have enabled the sonographic finding of fetal echogenic bowel (FEB), a sign that has been associated with various pathologies. Based on the experience of Brittany (western France), this study reports the type and frequency of the pathologies diagnosed

in fetuses with FEB, emphases on the prevalence of cystic fibrosis (CF) and on the carrier rate in that population, and assesses the ability of ultrasonography to detect CF *in utero*.

**Methods:** We reviewed all the consecutive cases of FEB diagnosed in pregnant women living in Brittany and who were referred for an analysis of the gene responsible for CF (*CFTR*) over the period 1992-2007. **Results:** Over that period, 289 diagnoses of FEB were recorded. A pathology was evidenced in 32.2% of the fetuses. Beyond CF (8.0%), it mainly included digestive tract malformations (7.0%), chromosomal abnormalities (3.7%), viral infections (3.7%) and cardiac disorders (2.9%). Regarding CF, we identified 23 CF fetuses and 19 heterozygous ones, leading respectively to a CF prevalence of 8.0% (1/13) and a carrier rate of 6.6% (1/15) in fetuses with FEB (rates significantly higher than in the general population). Moreover, by combining these data with those of our newborn screening programme, we showed that ultrasonography enabled to diagnose *in utero* 10.7% of the CF fetuses over the study period.

**Conclusion:** This study highlights the importance of ultrasound examinations of pregnancies and of the diagnosis of FEB, as well as the efficiency of ultrasonography to detect CF *in utero*.

#### P05.41

#### Walker-Warburg syndrome with hyperplastic primary vitreus detected by prenatal ultrasonography: case report

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Walker-Warburg syndrome (WWS) is an autosomal recessive disease presenting with muscular dystrophy associated with cerebral, cerebellar and eye anomalies, in which the life span of patients is short<sup>1</sup>. Following the detection of hydrocephaly during routine USG for a 28-weeks-pregnant mother, antenatal USG in the perinatology department revealed hydrocephaly along with persistent hyperplastic primary vitreous (PHPV). A presumptive diagnosis of Walker-Warburg syndrome was made. The information was used to treat this patient in her immediate postnatal life. This case, being the second in the literature with persistent hyperplastic primary vitreous (PHPV) detected by prenatal USG, demonstrates the importance of antenatal diagnosis in this condition.

#### P05.42

#### Sex reversal and growth retardation : two case reports with partial Xp duplication analyzed by array-CGH

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Transcription factors such as SRY, SOX9, DAX1, WT1, SF1 and WNT4 are involved in many genital development. Segmental duplication of the X chromosome can interfere with the testis differentiation in 46,XY subjects. The Xp region causing sex reversal is designated Dosage Sensitive Sex reversal and contains the DAX1 gene (Xp21.2).

We report two cases of females with growth retardation, facial dysmorphism, 46,XY karyotype and partial Xp duplication due to autosomal translocation. Such observations have been rarely reported. In both cases, Y chromosome and SRY gene were normal as ascertained by FISH and molecular biology.

Case 1 : cytogenetic analysis was motivated by growth retardation and hydramnios. Xp disomy was derived from a translocation with the 1 chromosome : 46,XY,der(1)t(1;X)(p36;p21). Fetal postmortem showed female external and internal genital tract with hypoplastic ovaries and hypoplastic adrenals. Under microscope, no tunica albuginea could be seen and the sex cords were dysgenetic. No primordial follicle were differentiated.

Case 2 : the female infant was born with severe growth retardation and ambiguous genitalia. She died of meningitis at 18 days of life. A Xp disomy was detected derived from a translocation with the 14 chromosome : 46,XY,der(14)t(14;X)(q32;p21).

Array-CGH analysis characterized the length of Xp duplications : 33.88 +/- 1.40Mb and 28.68 +/- 0.25Mb (case 1 and 2 respectively).

According to these results, two copies of *DAX1* were present in case 1 and could explain the fetal sex reversion, but there was only one copy in case 2. Other genes than *DAX1* could be implicated.

#### P05.43

#### Extreme nephromegaly: an unusual antenatal presentation of Zellweger syndrome

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Zellweger syndrome (ZS) is a rare severe autosomal recessive disorder, characterized by the reduction or absence of peroxisomes in liver, kidneys, and brain. ZS patients display dysmorphic features, severe neurological dysfunction, hepatic and renal failure, and rarely survive their first year.

Because of genetic heterogeneity, ZS diagnosis is usually based on biochemical investigation characterized by elevated plasma very-long-chain fatty acids (VLCFA), pipecolate and bile acid intermediates contrasting with low plasma plasmalogens. Some impaired enzymatic activity, such as dihydroacetone-phosphate acyltransferase deficiency (DHAP-AT) can also be detected in fibroblasts of ZS patients.

Here, we report on major and evolutive nephromegaly in a fetus with ZS. This was the first pregnancy for the healthy unrelated parents. Isolated nephromegaly (39mm, normal: 26mm) was observed at 22 weeks'gestation (WG). Clinical course was marked by a rapid increase in kidney size at 28 WG (68mm, normal: 32mm) with multiple cortical microcysts.

Diagnosis was performed after termination of pregnancy based on dysmorphic features (high forehead, macroglossia), nephromegaly without hepatomegaly and stippling of patella and hips.

Neuropathological findings included cortical polymicrogyria, cerebellar and bulbar heterotopias.

Biochemical investigations confirmed the deficit of the beta-oxydation of VLCFA and impairment of DHAP-AT enzymatic activity in cultured amniocytes.

To our knowledge, this is the first report of prenatal evolutive nephromegaly leading to diagnosis of Zellweger syndrome. We suggest that ZS should be considered as a possible diagnosis in evolutive nephromegaly.

#### P05.44

#### Growth and medical outcome of hundred and two 2-year-old children conceived after preimplantation genetic diagnosis or screening

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**Objective:** In preimplantation genetic diagnosis (PGD) or preimplantation genetic screening (PGS), embryo biopsy is an invasive essential procedure. The major objective of this study was to determine if the embryo biopsy might cause growth restriction and/or affect health outcome of children.

**Study design:** In this prospective study, 102 children (70 singletons and 32 twins) born after PGD/PGS were compared with a matched group for gender, maternal educational level, mother tongue and birth order of 102 children (70 singletons and 32 twins) born after intracytoplasmatic sperm injection (ICSI). Auxological data at birth and 2 years, physical findings, data on sociodemographic parameters, medical history of the pregnancy and the child were compared for both groups.

**Results:** No statistically significant differences regarding weight, height and head circumference standard deviation scores (SDS) at birth and at age two years were observed. At two years of age the mean body mass index SDS tended to be lower in PGD/PGS children ( $p=0.058$ ). PGD/PGS babies had been more often breastfed ( $p=0.013$ ), but mostly during a shorter time.

No difference was observed between both conception groups in the intake of medication and alcohol, cigarette smoking habits and complications during pregnancy. The prevalences of major as well as minor congenital anomalies, hospital admissions and surgical interventions were similar.

Conclusion: Children born after embryo biopsy applied in PGD/PGS present similar growth and physical health in the first two years of life as compared to ICSI children. These reassuring findings should be further confirmed in a larger cohort study.

#### P05.45

#### Assessing microsatellite instability in human preimplantation embryos

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Introduction: Mutations or silencing of genes involved in DNA mismatch repair pathways can result in microsatellite instability (MSI), a situation whereby repetitive DNA sequences are unstable during DNA replication. MSI is a common feature of many human tumours, whereas microsatellite mutations have also been reported in human spontaneously aborted fetuses. Couples undergoing Preimplantation Genetic Screening (PGS) are at high risk of meiotic and mitotic chromosome abnormalities. This study aims to investigate the level of MSI in this group of patients.

Materials/Methods: Fifty four blastomeres from 23 untransferred embryos from 5 PGS couples were subjected to whole genome amplification by Multiple Displacement Amplification (MDA). Genomic DNA and single lymphocytes from both parents were subjected to MDA and analysed using microsatellite markers in order to ensure that MDA did not introduce any artefacts. Embryonic and parental samples were analysed using the BAT26 and D5S346 markers and the embryonic haplotypes were compared to the corresponding parental patterns. The generation of any novel microsatellite alleles in the embryonic samples was interpreted as MSI.

Results: Out of the 54 cells isolated, 44 generated an MDA product. The BAT26 and D5S346 PCR results of these MDA products identified 2/23 embryos in which a novel allele was detected.

Discussion/Conclusion: This preliminary data suggests that MSI may be present in embryos from PGS cycles and further analysis is warranted. Comparison with MSI levels in embryos from PGD cases without fertility problems would elucidate whether embryos at high risk of aneuploidy are at increased risk of MSI.

#### P05.46

#### Is nuclear transfer morally acceptable as a means to prevent mtDNA disorders if it cannot avoid residual health risks?

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Since the 1990s, different variants of nuclear transfer (NT) techniques have been proposed as possible strategies for preventing the transmission of a mitochondrial DNA (mtDNA) mutation from mother to child. The aim is to provide prospective parents with genetically related offspring without the mtDNA mutation. In a clinical application of NT, it seems difficult if not impossible to avoid small amounts of affected mtDNA to come along with the oocyte, pronuclei or nucleus from the recipient woman. This potential carryover may result in the co-existence of two populations of mtDNA, also called mtDNA heteroplasmy. As long as it is not possible to carry out the procedure without the accompaniment of small amounts of affected mtDNA, the resulting embryo will always have a certain amount of mtDNA heteroplasmy. Although in many cases this will be far below the threshold to disease expression, the occurrence of mitochondrial disease symptoms cannot be ruled out completely, especially not in the resulting child's offspring: due to the existence of a genetic bottleneck, the mutant load may rise again in the third generation - the couple's grandchildren.

We will firstly discuss whether NT is morally acceptable as a means to prevent mtDNA disease if it cannot avoid residual health risks. Secondly, we discuss the proportionality of the procedure and compare NT with alternative reproductive options. Thirdly, as mitochondria are transferred maternally, male offspring will not pass on the mtDNA mutation. We examine whether this provides a reason to only create or transfer male embryos.

#### P05.47

#### Nuclear and mitochondrial genetic analysis in men opting for art

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Azoospermia factor (AZF) microdeletions, cytogenetic abnormalities and mtDNA mutations are the major genetic suspects of idiopathic male infertility. Therefore the aim of the study was to study genetic alterations (cytogenetic, Yq microdeletions, and mtDNA mutations) in infertile men with idiopathic infertility. Mt mutations were only assessed in men with idiopathic asthenozoospermia. DNA from semen was isolated from both patients and controls for AZF microdeletion analysis and mitochondrial genome was sequenced by standard PCR-DNA sequencing protocol. Of 1500 cases of infertility analysed 17% harboured cytogenetic abnormalities, 9.8% harboured AZF microdeletions which were found in idiopathic and non idiopathic cases. Mt mutations were analysed in 40 asthenozoospermic men. 72.5% (29/40) of the infertile patients showed significant nucleotide changes in their sperm mtDNA compared to the controls. Out of 70 infertile and 40 control samples analyzed, 80% infertile men were found to have significantly ( $p<0.0001$ ) high ROS levels in the semen compared to control. Thus genetic factors are an important aetiological factor which leads to irreversible spermatogenic arrest. mtDNA alteration may impair sperm motility both by depleting ATP and by increasing reactive oxygen species production. Increased ROS activity in the semen may be responsible for the nucleotide change in the mtDNA and for impairment of sperm motility. Our study highlights the need to analyze mitochondrial and nuclear genome in sperm samples for mutations and to assess the free radical levels in order to have better insight into pathophysiology of infertility. ICMR and AIIMS, New Delhi have partially supported the work.

#### P05.48

#### When should PGD be performed? A suggested scoring system for decision-making

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Introduction: PGD was designed to prevent the birth of offspring affected with severe genetic disorders. However, it may also be employed to prevent late onset and relatively mild conditions. Thus, clinicians find often uncertainty to decide whether a condition merits PGD.

Aim: To devise a scoring system that appraises the justification for PGD.

Methods: In order to construct the model we reviewed our PGD population for monogenic disorders. These include severe and early onset diseases, as well as conditions with late onset and incomplete penetrance. The scoring values (0-3) of the disease characteristics and patient variables were determined based on multi-disciplinary aspects.

Results: For each patient the parameters were summed resulting in values that ranged between 4-15 and the value of 9 was determined to be the threshold value for PGD suitability. Re-evaluation of our 106 patients that have already undergone PGD revealed that 100 of them (94.4%) scored with a value  $\geq 9$  and indeed suited for PGD. Though, 6 patients (5.6%) scored under the threshold value. This analysis was found to be congruent with the majority of our clinical decisions and reflected our policy.

Scoring system							
Onset	Severity	Penetrance	Risk for affected offspring	Infertility requiring IVF	PGD performed for other condition	Objection to T.O.P	Scoring value
Childhood/ congenital	Lethal	75-100%	-	-	-	-	3
Teen	Severe	50-75%	>25%	Yes	Yes	-	2
Early adulthood	Debilitating	25-50%	10-25%	-	-	Yes	1
Late adulthood	Mild	<25%	<10%	-	-	-	0

Conclusion: This scoring system is a useful tool in decision-making for patients' inclusion into a PGD program, enabling a consistent and

objective case management. It is especially valuable in genetic diseases with low penetrance, late onset conditions and cancer predispositions.

#### P05.49

##### Medical outcome of children born after PGD for HLA matching

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PGD (Preimplantation Genetic Diagnosis) with HLA (Human Leukocyte Antigen) matching allows to select unaffected, HLA compatible embryos to cure an affected sibling. Data on 27 pregnancies conceived after PGD-HLA between 2004 and 2008 are reported. Children were examined at the age of 2 months, 1 and 2 years at our centre to analyse the medical outcome.

The PGD-HLA procedure was applied to 175 cycles for 65 couples. Indications were Beta thalassemia, Severe Combined Immune Deficiency syndrome, Wiskott Aldrich syndrome, Fanconi Anemia, Acute Lymphatic Leukemia, Sickle cell anemia, Duncan's syndrome, Acute Myeloid leukemia, hyper IgM syndrome, Diamond-Blackfan anemia. These treatments resulted in 79 transfers, 27 pregnancies (3 biochemical, 5 miscarriages, 5 still ongoing) and 14 deliveries (1 twin, 13 singletons). Outcome data are missing for one child. Four boys and 10 girls had a mean birth weight of  $3256 \pm 637$  grams (2300 to 4650 gr) at a mean term of  $38.3 \pm 1.6$  weeks (36 to 42 weeks). One girl had a left eye congenital cataract. Three siblings underwent a successful transplantation of haematopoietic cells. One affected child died before the birth of the HLA compatible sibling. Another ill child was transplanted with bone marrow from an allogenic donor while awaiting the birth of the HLA compatible sibling. The other affected children have as yet not been transplanted.

Children born after PGD-HLA appear to be in good health. These preliminary data will be further evaluated in a larger long-term follow-up study including medical and psychological outcome of child and family.

#### P05.50

##### Polar body diagnosis for female translocation carriers applied during conventional IVF treatment

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**Objective:** Carriers of reciprocal and Robertsonian translocations are faced with increased reproductive risks depending on the chromosomes which are involved in the chromosomal rearrangements. According to the restrictive legal situation in Austria, preimplantation genetic diagnosis is limited by law to polar body diagnosis (PBD). Polar body diagnosis was therefore offered and used to identify balanced/normal and unbalanced oocytes.

**Materials/Methods:** Polar body diagnosis using FISH (fluorescence in-situ hybridisation) was performed for 9 couples with the female carrying a translocation during conventional IVF cycles. We used translocation specific combinations of distal and proximal probes to the breakpoint, which allowed differentiation of unbalanced and balanced oocytes.

**Results:** Polar body diagnosis was performed for 14 cycles of translocation diagnosis (7 cycles for reciprocal; 7 cycles for Robertsonian translocations).

After PBD only 19 % of oocytes were diagnosed as normal/balanced and 81 % showed an unbalanced chromosomal content. From 14 cycles performed, only 4 cycles (28,6 %) had euploid oocytes available, 71,4 % showed solely aneuploid oocytes.

**Conclusion:** In this study, we found a higher than average aneuploidy rate in oocytes of female translocation carriers. Interestingly, patients with Robertsonian translocations showed more euploid oocytes than carriers of reciprocal translocations. The high rate of chromosomally unbalanced oocytes lead to the conclusion, that for carriers of maternal translocations who already need IVF treatment for infertility, polar body diagnosis is a valuable additional selection tool. Screening for imbalance in cases with increased risk of viable chromosome abnormality might help to decrease miscarriages and pregnancy termination after prenatal diagnosis.

#### P05.51

##### Preimplantation Genetic Diagnosis: International Regulatory Approaches

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In the last decade, innovations in reproductive genetic testing and human genomic research have altered the policy and legal approaches surrounding reproductive choices. Innovations in both prenatal diagnosis (PND) and preimplantation genetic diagnosis (PGD) provide prospective parents with information regarding phenotypic or genetic traits, health status and the sex of their offspring. This information has important implications for reproductive decision-making yet, reproductive choices must be weighed against broader ethical, legal or social issues particular to each diagnostic procedure. Contradictory views and opinions abound in different countries creating an inconsistency in the manner in which PND and PGD are regulated. This is due to the presence or absence of universal health care systems and to demographic, cultural or socio-economic factors that underlie different regulatory approaches. The aim of this article is to provide an analysis of current laws and policies regarding PND and PGD and the regulatory trends in sixteen different countries: Australia, Belgium, Canada, China, France, India, Israel, Japan, Netherlands, New Zealand, Singapore, Switzerland, South Africa, Spain, United Kingdom and the United States. Our analysis shows that, despite the similarities between PND and PGD in terms of their uses and outcomes, PND is progressively considered part of routine prenatal care while the various uses of PGD, although expanding, are being heavily scrutinized resulting in the tendency for greater regulatory oversight by the majority of countries surveyed. Such scrutiny requires making explicit both the reasons for additional indications for PGD and the justifications for prohibitions, if any.

#### P05.52

##### Preimplantation Genetic Diagnosis for complex chromosome rearrangements

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Chromosomal rearrangements are relatively common: around 1 individual out of 1000 is carrying a Robertsonian translocation, 1/1000 a balanced reciprocal translocation, 1/10000 an inversion.

The probability of having a couple where both member is carrying such an abnormality is around 4.4 by million, i.e. around 50 couples in age to procreate in France.

Preimplantation Genetic Diagnosis (PGD) is offered to patients having a risk to transmit a genetic disease to their offspring.

PGD is frequently used for single chromosomal rearrangement, using fluorescence in situ hybridisation techniques (FISH) on embryo's cell nuclei.

In case of complex chromosomal rearrangement (CCR) PGD faces two major difficulties, I) the chances of having an unaffected embryo are low, II): technical difficulties are increased to diagnose all possible unbalanced karyotypes in embryos.

Two couples having PGD for CCR are presented here:

- Couple 1: 45,XY,der(14;21)(q10;q10) and 46,XX,inv(10)(p13q11.2)

- Couple 2: 45,XY,der(15;21)(q10;q10) and 46,XX,t(1;9)(q25;q34.1)

Chances for having balanced embryos were estimated as sufficient to offer PGD to these couples.

We described here the development of specific FISH techniques for these PGDs, allowing an accurate analysis in a short time compatible with the necessity to rapidly replace embryos into uterus.

PGD resulted in pregnancy for each couple, but one terminated in spontaneous abortion.

This work supports the idea that PGD can be efficiently offered to couples with complex chromosomal indications if I) the proportion of expected balanced embryos is sufficient and II) a reasonable number of oocytes can be obtained by ovarian stimulation.

**P05.53****Rapid prenatal of chromosome aneuploidy by QT-PCR***J. Kasnauskiene<sup>1,2</sup>, N. Krasovskaja<sup>2</sup>, V. Kučinskas<sup>1,2</sup>;**<sup>1</sup>Vilnius University, Vilnius, Lithuania, <sup>2</sup>Vilnius University Hospital Santariskiu Clinics, Vilnius, Lithuania.*

QT-PCR can detect the great majority of chromosome abnormalities in prenatal samples being targeted to chromosomes 13, 18, 21 X and Y. It is a rapid robust and accurate test for the prenatal diagnosis of most frequent trisomies. Main advantages of the test: low cost, speed and automation large scale application. In Lithuania QT-PCR is as a stand-alone test for a sub-set of prenatal samples, with the aim of minimising the identification of chromosome abnormalities of unknown clinical significance and reducing double testing. All prenatal samples are tested for trisomies 13, 18, 21, X and Y chromosomes using QT-PCR. Karyotype analysis is carried out only for referrals with NT >3 mm before 14 weeks or >6 mm thereafter, ultrasound abnormalities (excluding single soft marker), from families with a previous chromosome abnormality (excluding single soft markers). During four years 1187 AF and 24 CVS were received. AF 22 % were karyotyped. 4.8 % AF and 4.2 % CVS were abnormal. All aneuploidies involving chromosomes 13, 18, 21, X and Y were detected with sensitivity and specificity 100%. Large scale application of QT-PCR has reduced the load of prenatal cytogenetics if all pregnancies are monitored by non invasive methods.

**P05.54****Molecular karyotyping of whole genome amplified samples***H. Raussi<sup>1</sup>, M. Mäkinen<sup>2</sup>, S. Ruosaari<sup>1</sup>, A. Godenhjelm<sup>1</sup>, P. Ollikka<sup>1</sup>;**<sup>1</sup>PerkinElmer Human Health, Turku, Finland, <sup>2</sup>University Of Turku, Turku, Finland.*

Background: Array comparative genomic hybridization (array CGH) has been widely used for detection of copy number changes in tumors and genetic disorders. In the prenatal setting, however, where starting DNA amounts may be too low for array CGH, the feasibility of the technology remains to be defined. Although whole genome amplification (WGA) has potential for expanding the use of array CGH in analyses of few nanograms of DNA or even single cells, further information is needed about the putative bias WGA introduces to the genomic profiles.

Methods: We performed WGA on DNA samples and analyzed the resulting genomic profiles by means of array CGH. The amplification was performed with GenomePlex® (SigmaAldrich) products using few nanograms of DNA or few cells as a starting material. To assess possible bias introduced by WGA, the amplified test samples were hybridized using both the respective native and an amplified reference DNA as the reference. PerkinElmer's Constitutional Chip® platform and SpectralWare® analysis software were used in the array CGH analysis.

Results and conclusions: Our results indicate that the Constitutional Chip® platform works effectively for generating array CGH data from amplified test and reference samples. However, when native DNA is compared against the respective amplified DNA, specific chromosome regions continuously fail to amplify, especially as the amount of the starting material is decreased. The results show that detection of aneuploidies is not hampered by the sequence drop-out, yet micro-level changes may be missed owing to the biased amplification.

**P05.55****Detection of fetal trisomies with a 19-plex QF-PCR using the novel AmpliTaq Gold PCR 360 Master Mix***R. Achmann<sup>1</sup>, F. Stellmer<sup>1</sup>, K. Held<sup>1</sup>, H. Schulte<sup>1</sup>, A. Sartori<sup>2</sup>;**<sup>1</sup>MVZ genteQ, Hamburg, Germany, <sup>2</sup>Applied Biosystems GmbH, Darmstadt, Germany.*

In recent years quantitative fluorescent polymerase chain reaction (QF-PCR) with short tandem repeat markers (STR) have become the method of choice for the prenatal detection of the most common chromosome aneuploidies. The main advantages of QF-PCR are its accuracy and speed. Typically test results can be obtained within a few hours after sample receipt.

We established an elaborated multiplex QF-PCR covering the chromosomes 13, 18, 21 and X with a total of 19 polymorphic markers. Primers for amplification of markers were labeled with four different fluorescent dyes. PCR products were analyzed in parallel on a multicapillary electrophoresis instrument. We compared results obtained with our

standard protocol using a chemically blocked hot start Taq polymerase with Applied Biosystems novel AmpliTaq Gold PCR 360 Master Mix. We were able to reduce cycling time from 2:45 hours to 2 hours with AmpliTaq Gold PCR 360 Master Mix without compromising the PCR yield, specificity and sensitivity. We observed reliable amplification of marker alleles allowing us to correctly deduce fetal chromosomal aneuploidies from amniotic fluid and 'chorionic villus' samples.

**P05.56****Quantitative analysis of foetal DNA in maternal circulation in gestational diabetes mellitus (GDM) pregnancies***M. Zamanpoor<sup>1</sup>, T. Karuppiah<sup>1</sup>, R. Rosli<sup>1</sup>, M. Yazid<sup>1</sup>, Z. Husain<sup>2</sup>;**<sup>1</sup>Clinical Genetics Unit, Department of Obstetrics and Gynaecology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Malaysia,**<sup>2</sup>Department of Interdisciplinary Medicine, BIDMC, Harvard Medical School, Boston, MA, United States.*

Gestational Diabetes Mellitus (GDM) is a condition in which women without prior diagnosis of diabetes, show high levels of blood glucose during pregnancy. GDM affects 3-10% of pregnancies. Studies have shown that women with GDM have a higher risk of preeclampsia and that their offsprings are at a higher risk for congenital malformations. Increased amount of cell free foetal DNA (cffDNA) in maternal plasma has been found in adverse pregnancies such as preeclampsia, gestational hypertension, foetal chromosomal aneuploidies, placental abnormalities, preterm labour and hyperemesis gravidarum. It was suggested that elevation of cffDNA in maternal plasma could be used for early identification of adverse pregnancies. The aim of our study was to determine the elevation of cffDNA levels in GDM pregnancies compared to normal pregnancies. In this study, twenty-three second-trimester plasma samples from GDM pregnancies were compared with fourteen control samples of normal pregnancies, all carrying a singleton male foetus. The cffDNA concentrations were measured by quantitative real-time PCR amplification of SRY gene which is located on Y chromosome, using TaqMan dual labelled probe system. The mean of cffDNA levels for cases and controls were 3.47 and 5.64 genome equivalents/ml, respectively. No significant differences ( $P = 0.17$ ) in the mean of cffDNA concentration were observed between GDM and normal pregnancies. In conclusion, GDM does not affect levels of maternal plasma cffDNA. Hence, if cffDNA is used as an additional serum marker in prenatal screening test in the future, our data suggests that cffDNA quantity will not require adjustment for GDM pregnancies.

**P05.57****Evaluation of automated DNA extraction for non invasive prenatal diagnosis using free fetal DNA in maternal plasma***E. Ordoñez<sup>1</sup>, L. Rueda<sup>1</sup>, P. Cañadas<sup>1</sup>, C. Mediano<sup>2</sup>, C. Fuster<sup>3</sup>, V. Cirigliano<sup>1</sup>;**<sup>1</sup>General Lab, Barcelona, Spain, <sup>2</sup>Hospital Vall d'Hebrón, Barcelona, Spain,**<sup>3</sup>Universitat Autònoma de Barcelona, Barcelona, Spain.*

The use of free fetal DNA for non invasive prenatal detection of fetal sex and RhD status is part of the daily routine in several genetic centers. Automation of DNA extraction process simplifies sample handling allowing high throughput of samples. We evaluated the suitability of using an automated DNA extraction procedure for ffDNA extraction in comparison with the most commonly used manual method.

Fifty Blood samples collected in the first trimester of pregnancy from pregnant women with male fetuses were selected for this study. DNA extractions were performed by using 500µL of plasma for the QIAamp DSP Virus Kit (QIAgen Inc.) or 1mL of plasma for the COBAS AmpliPrep® DNA/RNA extractor from ROCHE. ffDNA was quantified by rtPCR amplification of the SRY gene and yield (GE/mL) was calculated for both extraction methods.

SRY amplification was detectable in all samples independently from the extraction procedure and no false negative results were observed. ffDNA quantity obtained by the manual extraction method was higher than using the automated extraction with a mean of 259,43GE/mL and 109,47GE/mL plasma respectively.

The lower ffDNA yield obtained using an automated DNA extraction procedure does not affect the efficiency of detecting fetal specific sequences in maternal plasma by rtPCR. Automated DNA extraction allows high throughput of samples (up to 72 samples/run) in a closed system thus greatly reducing the risk of cross contamination; it also requires less sample manipulation reducing overall costs and hands on time.

**P05.58****Partial submicroscopic duplications in markers D13S631 and X22 detected with QF PCR****R. Raynova, S. Andonova, J. Genova, I. Kremensky;****National Genetics Laboratory, University Hospital of Obstetrics and Gynecology, Sofia, Bulgaria.**

Rapid diagnosis by Quantitative Fluorescent PCR analysis (QF-PCR) has proved its cost-efficiency, speed and efficacy for prenatal detection of the most common autosome aneuploidies - trisomy 21, trisomy 18, trisomy 13. For the past three years in our laboratory QF-PCR was carried out on 2 300 prenatal samples (amniotic fluid, CVS). Fourteen polymorphic STR markers (4 located on chromosome 21, 4 - on chromosome 18, 3 - on chromosome 13, and 3 on chromosomes X and Y) were amplified with Cy5-labeled primers. Trisomy was indicated by at least two informative markers on a single chromosome, showing triallelic (1:1:1) or diallelic (1:2/2:1) trisomic pattern. In rare cases a single marker result, consistent with trisomy may be observed, whilst all other informative markers on the same chromosome are normal. A partial submicroscopic duplication could be suspected. We have found 9 samples to have a single marker result consistent with trisomy - 8 samples showed trisomic pattern for marker D13S631, one for marker X22. In cases when parental DNA was available we proved maternal or paternal origin of the duplication. Thus we could distinguish the rare inherited polymorphism from unbalanced translocation resulting to partial trisomy.

**P05.59****Large scale application of QF-PCR for rapid prenatal diagnosis of common chromosome aneuploidies, results of nine years clinical experience****V. Cirigliano<sup>1</sup>, G. Voglino<sup>2</sup>, E. Ordoñez<sup>1</sup>, P. Cañadas<sup>1</sup>, L. Rueda<sup>1</sup>, C. Fuster<sup>3</sup>, M. Adinolfi<sup>1</sup>;****<sup>1</sup>General Lab, Barcelona, Spain, <sup>2</sup>Promea-Day Surgery, Turin, Italy, <sup>3</sup>Universitat Autònoma de Barcelona, Barcelona, Spain, <sup>4</sup>University College London, London, United Kingdom.**

Despite being deliberately targeted to chromosomes 13, 18, 21, X and Y the rapid QF-PCR test can detect the great majority of chromosome abnormalities in prenatal diagnosis. The main advantages of the assay are low cost, speed and automation allowing large scale application. We developed a QF-PCR assay that was employed to test 43.000 clinical samples with results issued in 24 hours. The most common referral indications were raised biochemical risk (32%) and advanced maternal age (30%). All samples were also tested by conventional cytogenetic analysis and the results compared.

A total of 1550 non mosaic aneuploidies involving chromosomes 21, 18, 13 X and Y were detected with 100% specificity. Several cases of partial trisomies and mosaicism were also identified. Overall 95% of clinically relevant abnormalities were readily detected and termination of the affected pregnancies could be performed without waiting for results of the cytogenetic analyses. Our results support the possibility of reducing the load of prenatal cytogenetic tests if pregnancies are carefully monitored by non invasive screening. As a result, invasive procedures should only be performed in high risk pregnancies. In case of abnormal QF-PCR results, medical action can be made available in a few hours after sampling. In cases of negative QF-PCR results cytogenetic analyses might only be performed for fetuses with clear evidence of abnormalities based on ultrasound results. In Countries, where large scale conventional cytogenetic tests are hampered by high cost and lack of technical expertise, QF-PCR may be used as the only prenatal diagnostic test.

**P05.60****Rapid prenatal diagnosis by QF-PCR analysis on 700 cases****A. M. Stan<sup>1</sup>, C. Dragomir<sup>1</sup>, E. Severin<sup>2</sup>, L. Savu<sup>1</sup>;****<sup>1</sup>Genetic Lab S.R.L., Bucharest, Romania, <sup>2</sup>Carol Davila University of Medicine and Pharmacy, Bucharest, Romania.**

The quantitative fluorescence PCR (QF-PCR) assay was recently introduced in Romania in the prenatal diagnosis field allowing the rapid detection of most frequent chromosomal aneuploidies involving chromosome 21, 18, 13, X and Y in high-risk pregnancies.

The main objective of this study was to demonstrate that QF-PCR based assay is an efficient, reliable and rapid method that can reduce the number of conventional cytogenetic analysis in carefully monitored

pregnancies. Therefore we performed rapid prenatal testing for 700 cases proceeding from small amounts of fetal sample including amniotic fluid, chorionic villi and products of conception.

The methodology included the relative quantification of microsatellite alleles to determine sequence copy number, using a multiplex QF-PCR reaction and capillary electrophoresis. The multiplex QF-PCR reaction was performed with a commercial available kit for rapid prenatal diagnosis of trisomy 21, 18, 13 and sex chromosome aneuploidies.

We analyzed 675 amniotic fluid samples, 13 products of conception and 12 chorionic villi samples. All samples with trisomy 21 (n=17), trisomy 18 (n=3), trisomy 13 (n=1), trisomy XXY (n=2), monosomy X (n=2), triploidy (n=2) and mosaicism (n=2) were accurately diagnosed without false-negative results. We detected somatic microsatellite mutation in two cases and submicroscopic polymorphic duplications in four samples, all six cases involving a single STR marker. All six cases were reported as normal by cytogenetic analysis.

In conclusion we are confident that the QF-PCR approach is an efficient, reliable and rapid prenatal diagnosis method offering an alternative to conventional cytogenetic testing.

**P05.61****Analysis of heterozygosity level of STR markers by QF-PCR method for prenatal diagnostics in Northwest region of Russia****I. Belotserkovsky, G. Demin, T. Ivashchenko, I. Fedorova, V. Baranov;****Ott's Institute of Obstetrics and Gynecology RAMS, Saint-Petersburg, Russian Federation.**

Last years prenatal diagnosis for the most common chromosome abnormalities, such as Down's, Edward's, Patau's syndromes and also numerical sex chromosomes abnormalities has carried out by quantitative fluorescent PCR (QF-PCR). QF-PCR is highly sensitive and specific test revealing these chromosome abnormalities which account for around 95% of all chromosome pathologies of the fetus. We have used 11 chromosome-specific of markers (DXS6854, D13S628, D13S634, D13S742, D18S386, D18S380, D18S391, D18S535, D21S1270, D21S1411, D21S226) to determine the heterozygosity level for 203 DNA samples from Northwest region of Russia individuals. The lowest heterozygosity level was registered for D18S380 marker (59%) whereas the highest level was found 89% for D13S742 marker. The mean level of heterozygosity was found as 74%. When using two markers on the same chromosome heterozygosity level was increased: 99% - for chromosome 13; 91% - for chromosome 18 and 97% - for chromosome 21. Use of three and more markers on each chromosome increases level of heterozygosity and informativeness of the method up to 99-100%. Heterozygosity levels of D18S535 and D13S742 markers were significantly different from English population. We found that D13S742, D18S386 and D21S1411 markers are the most informative for prenatal diagnostics in Northwest region of Russia.

**P05.62****Quantitative real-time PCR technique for rapid and prenatal diagnosis of trisomy 21 syndrome****A. R. Kamyb<sup>1,2</sup>, N. Masroori<sup>2</sup>, F. Maryami<sup>1</sup>, R. Mirfakhraie<sup>3</sup>, F. Maryami<sup>1</sup>, M. Karimipoor<sup>4</sup>, R. Mahdian<sup>1</sup>;****<sup>1</sup>Department of molecular medicine, Pasteur institute of Iran, Tehran, Islamic Republic of Iran, <sup>2</sup>Department of biology, science and research branch, Islamic Azad university, Tehran, Islamic Republic of Iran, <sup>3</sup>Department of medical genetics, National institute of genetic engineering and biotechnology, Tehran, Islamic Republic of Iran.**

Down's syndrome (DS) is a genetic disorder that affects 1 in 700 live births across all ethnic groups. Trisomy 21 syndrome is caused by an extra copy of chromosome 21. Currently cytogenetic methods as karyotyping and FISH are used for diagnosis of DS. However these are labor and too time-consuming. On the contrary, molecular methods such as Real-Time PCR are sensitive and high throughput. According to the study, peripheral blood was collected from patient and normal controls. Subsequently by salting out method, genomic DNA was purified. In this study, two genes as DSCAM and DYRK1A2 (target genes) and PMP22 (reference gene) locating on chromosome 21 and 17 respectively, selected and measured copy number of them in trisomy 21 patients and normal individuals. Then, DSCAM/PMP22 and DYRK1A2/PMP22 ratio was calculated by  $2^{-\Delta\Delta Ct}$  formula. The results of Real-Time PCR showed the ratio of  $1.71 \pm 0.16$  and  $1.01 \pm 0.10$  (p

value<0.001) in DS and normal samples respectively, demonstrating 3 copies of target genes in trisomy 21 syndrome (DS) and 2 copies in normal while reference gene (PMPM22) were two copies in both samples. These results compared to the upshots of cytogenetic karyotype analysis. To put it briefly, Quantitative Real-Time PCR can be used as a sensitive, accurate and reliable technique for rapid and prenatal diagnosis of trisomy 21 syndrome.

## P06. Cancer genetics

### P06.001

#### 15-Lipoxygenase-1 (15-LO-1) in the proliferation, adhesion, migration and invasion of colorectal carcinoma cell lines

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Colorectal carcinoma (CRC) is often lethal when invasion and/or metastasis occur. 15-lipoxygenase-1 (15-LO-1), an enzyme involved in the oxidative metabolism of linoleic acid, is down-expressed particularly in CRC. However, little is known about the role of the enzyme in the process of tumor metastasis. In this study, we investigated the hypothesis that 15-LO-1 over-expression in CRC cells results in decreased cell proliferation, adhesion, migration and invasion and increased apoptosis. We have over-expressed 15-LO-1 in HCT-116 and HT29 cells by the use of stable and transient transfections respectively with the eukaryotic expression vector pcDNA3.1-15-LO-1. Cellular proliferation was analysed by MTT assay and the apoptotic potential of 15-LO-1 was evaluated by acridine orange and caspase-3 assays as well as expression levels of the antiapoptotic protein XIAP. Cellular migration and invasion were investigated by scratch wound healing assay, adhesion on fibronectin, Boyden chamber migration assay as well as Matrigel invasion assay. Our data indicate that over-expression of 15-LO-1 in CRC cell lines significantly decreased cell proliferation, adhesion, migration and invasion, and significantly increased apoptosis. These results suggest that 15-LO-1 expression in CRC can inhibit colon cancer cell growth through induction of apoptotic cell death and may contribute to the inhibition of their invasive and metastatic capacity *in vitro*.

### P06.002

#### Somatic selection of the 8q24 risk allele in colorectal cancer

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The presence of risk allele for the development of sporadic colorectal cancer (CRC) at 8q24 (rs10505477) was suggested, although the exact mechanism responsible for this trait has not been determined yet. We evaluated whether this locus is involved in the somatic evolution of the disease by selection of the risk variant in tumors. We analyzed paired blood and tumor samples from 59 heterozygote CRC patients using Real-time PCR allele discrimination method. The overall frequency of allelic imbalance (AI) was 27%, of which 62.3% had preferential selection of the risk allele. Although the number of analyzed samples was rather small, we couldn't observe any difference in age of onset, sex, localization, stage at diagnosis or MSI status between patients with or without AI at 8q24. To determine the mechanism of AI, we analyzed the allelic content at 8q24 in additional 20 homozygotes for either the risk A or the wild-type G allele using the NAT2 gene as internal reference. Using this approach we found that 8 samples had a selection of the A allele (5 due to the amplification and 3 due to the loss of G allele) and 3 samples had selection of the G allele (2 due to the amplification and 1 due to the loss A allele). Based on our data we conclude that the risk allele at 8q24 is preferentially selected in tumors of colorectal cancer patients, with no preferential mechanism (amplification or the risk allele or loss of the wild-type allele) responsible for this trait.

### P06.003

#### Opposing roles of telomerase in the generation of polyploidy during continuous neoplastic cell growth

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Abnormal chromosomal segregation is a process of tumor genome evolution that occurs frequently in all types and stages of human neoplasia. Whole Genome Duplication (WGD) is a common way to create polyploid cells and possibly occurs in response to cellular DNA damage responses. Although WGD has been observed during telomere crisis in hyper-proliferating cells and telomerase deficient mouse embryo fibroblasts (MEFs), telomere dysfunction in cancerous growth has been mainly related to structural chromosomal instability. Our data obtained from a panel of telomerase positive and negative human immortalized cell lines show that the telomere defective Alternative Lengthening of Telomeres (ALT) is characterized by significantly higher rates of *in vitro* WGD as compared to telomerase positive growth. Furthermore, telomere dysfunction after depletion/inhibition of hTERT in telomerase positive cell lines was accompanied by significant increase in the frequencies of WGD. In this context, reconstitution of telomerase activity resulted in suppression of WGD. To test the generality of these findings we examined WGD in the ALT VA-13 cells containing a constitutive hTERC and a tet-inducible hTERT. When telomerase activity was induced in these cells there was an immediate increase in the rates of dysfunctional telomeres and WGD. These results are consistent with the view that the generation of WGD occurs gradually during telomere dysfunction and can be triggered to be massive in a crisis interphase state between different types of telomere maintenance. Our data suggest a telomere-related universal mechanism of WGD in neoplasia. In this process telomerase activity has both enhancing and suppressive roles.

### P06.004

#### Crosstalk between T-cadherin and Wnt/β-catenin pathways in cancer

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Adiponectin induces PI3K/Akt and Wnt/β-catenin pathways which regulate the growth and survival of cancer cells, it works through its cell surface membrane receptors AdipoR1, AdipoR2 and T-cadherin. We studied the expression level of adiponectin and its receptors in 30 pediatric acute myeloid leukemia (pAML) patients and a group of solid tumors (n=40) with tumor free control samples by qRT-PCR. The expression of the adiponectin was not detected in pAML patients and solid tumors in spite of the significant expression of its two major regulators; TNF-α and IL-6, when compared to CD33+ blasts from healthy controls and tumor free controls, respectively. The expression of AdipoR1 was observed to be lower in pAML patients and in breast and stomach tumors compared to controls. AdipoR2 expression was found to be higher in pAML patients compared to controls. In tumor tissues AdipoR2 expression was similar to AdipoR1. T-cadherin expression was lower in breast and colon tumors but higher in pancreas and stomach tissues. Interestingly, T-cadherin expression was significantly higher in pAML patients compared to controls ( $p<0.01$ ). T-cadherin/β-catenin complex together with the controlling proteins APC and GSK-3β were also studied in pAML. Interestingly, β-catenin expression was found to be significantly higher compared to controls whereas APC expression was not detected and GSK-3β was higher than the controls. Increased expression of T-cadherin and β-catenin expression might indicate their possible role in cell-cell adhesion in pAML. The cross-talk between T-cadherin and β-catenin need to be further investigated in order to understand the interaction between PI3K/Akt and Wnt/β-catenin signaling pathways in the progression of cancer.

**P06.005****Tiling resolution array-based comparative genomic hybridisation analyses of acute lymphoblastic leukaemias in children with Down syndrome reveal recurrent gain of 8q and deletions of 7p and 9p****C. Lundin<sup>1</sup>, J. Davidsson<sup>1</sup>, L. Hjorth<sup>2</sup>, M. Behrendtz<sup>2</sup>, B. Johansson<sup>1</sup>**<sup>1</sup>Dept of Clinical Genetics, Lund, Sweden, <sup>2</sup>Dept of Pediatrics, Lund, Sweden,<sup>3</sup>Dept of Pediatrics, Linköping, Sweden.

Down syndrome (DS) is a risk factor for childhood acute leukemia, with a cumulative risk of 2.1% at the age of 5 years. Acute lymphoblastic leukemia (ALL) is the most prevalent form, constituting 60% of new cases.

A total of 286 pediatric B-ALLs have been cytogenetically analyzed at our department. Ten (3.5%) were DS-ALL and DNA was available for array CGH analysis in seven of these. Using G-banding, acquired changes were detected in five of the cases. None of these aberrations were recurrent. However, by the use of the 32K platform, the following recurrent genomic imbalances were observed: del(2)(p11.2p11.2), gain of 8q and deletions of 7p and 9p.

The minimally deleted region in 7p occurred at 7p12, which was lost in two of our cases as well as in three DS-ALLs from the literature. Regarding the 9p deletions, the focal deletions of *CDKN2A* and *PAX5* previously reported might well correspond to the deletions encompassing 9p11.2-22.3 in two of our cases and the common deleted segment 9p13-p22 in two further cases of DS-ALL recently published. An intriguing finding of the present study was a deletion of 12q in one case, comprising *KITLG* in 12q21.3. In adults, this gene has been described to function in hematopoiesis.

Thus, some interesting regions and specific genes have recently been identified in acute leukemias in children with Down syndrome and more high resolution studies are clearly needed to evaluate these findings and to clarify further the molecular genetic picture in this patient cohort.

**P06.006****Investigation the correlation of Phenotypes with the Genotypes of the AAT in Esophageal Squamous Cell Carcinoma (SCCE)****S. Mohammad Ganji<sup>1</sup>, F. Rastgar-Jazi<sup>1</sup>, A. Sahebghadam-Lotfi<sup>1,2</sup>, M. Yazdanbod<sup>3</sup>, A. Mota<sup>2</sup>, A. Mohsenifar<sup>2</sup>**<sup>1</sup>NIGEB, Tehran, Islamic Republic of Iran, <sup>2</sup>Biochemistry Department, Tarbiat moddares University, Tehran, Islamic Republic of Iran, <sup>3</sup>Madaen Hospital, Tehran, Islamic Republic of Iran.

Proteolytic enzymes such as Alpha-1 antitrypsin (AAT) play significant role in malignancy including Loss of growth regulation, invasiveness and metastases. AAT is the most abundant serin protease inhibitor in human plasma which produced mainly in liver and monocytes and deficiency of it, is an inherited disorder characterized by reduced serum level of AAT. The most common deficient genotypes of AAT are Protease inhibitors Z(PiZ) and S(PiS). The association of deficient AAT subtypes with several tumors such as primary liver carcinoma, lung cancer, gastrointestinal cancer and malignant hepatoma was reported. This study aims to test relationship between AAT phenotypes and genotypes Z and S with Esophageal Squamous Cell Carcinoma (SCCE) cancer in patients whom were attending Madaen Hospital, in Tehran, during 2006-2007. Serum and DNA were isolated from 37 patients and Isoelectricfocusing was carried out on serum samples. Genotypes of S and Z alleles were performed by RFLP technique using *TaqI* restriction enzyme. Our results indicated that the mean range for TIC and AAT by nephelometry test of patients were significant difference rather than healthy peoples ( $P<0.05$ ). Moreover, 97.3% of the esophageal cancer patients were homozygote for the normal allele protease inhibitor MM(PiMM), while 2.7% were MS heterozygote and no PiZ and PiS genotypes were found ( $P>0.05$ ). In conclusion, we can report a relationship between AAT deficient genotypes S and esophageal cancer in the studied patients.

**P06.007****Mutations of Hh-Gli Signaling Pathway Genes in Basal Cell Carcinomas (BCCs)****V. Musani<sup>1</sup>, M. Cretnik<sup>1</sup>, M. Levacic Cvok<sup>1</sup>, P. Ozretic<sup>1</sup>, M. Situm<sup>2</sup>, D. Leovic<sup>3</sup>, S. Levanat<sup>1</sup>**<sup>1</sup>Rudjer Boskovic Institute, 10002 Zagreb, Croatia, <sup>2</sup>Department of Dermato-venerology, Clinical Hospital "Sestre milosrdnice", University of Zagreb, 10000

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Basal cell carcinoma (BCC) of the skin is the most common human cancer, and shows a continuously increasing incidence. BCCs occur predominantly on sun-exposed skin of elderly fair-skinned people. Several tumor suppressor genes and oncogenes have been implicated in the pathogenesis of BCCs, most of them being members of the Hedgehog-Gli (Hh-Gli) signaling pathway.

Hh-Gli signaling pathway is one of the fundamental signaling pathways in embryonic development and is required in vertebrates for normal development of many structures, including the neural tube, axial skeleton, skin and hair.

Normally, a secreted protein Hedgehog (Hh) binds to a transmembrane protein Patched (Ptch), which causes release of another transmembrane protein Smoothened (Smo) from its repression by Ptch. This triggers a signaling cascade in the cytoplasm which leads to translocation of the activated transcription factor Gli to the nucleus and expression of target genes (Gli, Ptch, WNT and TGF beta family members, various cyclins), involved in cell proliferation, while Ptch acts as a limiting factor by blocking pathway activity.

Aberrant activation of the pathway in adult tissue is associated with the development of various tumors (BCC, medulloblastoma, pancreatic, gastrointestinal etc.).

In our research we investigated mutation status of several Hh-Gli pathway genes in sporadic BCCs. The mutation screening was performed by high resolution melting approach, which is based on differences in melting curves caused by variations in nucleotide sequence; detected variants were confirmed by direct sequencing.

Our results show mutations in several pathway members (SHH, PTCH, SUFU) implicating their involvement in tumor pathogenesis.

**P06.008****The intracellular localization of folliculin (FLCN) in Birt-Hogg-Dubé syndrome****M. Vreeburg<sup>1</sup>, T. Claessens<sup>2</sup>, D. Marcus-Soekarman<sup>1</sup>, M. van Geel<sup>2</sup>, M. A. M. van Steensel<sup>2,3</sup>**<sup>1</sup>University hospital Maastricht, department of clinical genetics, Maastricht, The Netherlands, <sup>2</sup>University hospital Maastricht, department of dermatology, Maastricht, The Netherlands, <sup>3</sup>Research Institute Growth & Development (GROW), University Maastricht, Maastricht, The Netherlands.

Birt-Hogg-Dubé syndrome (MIM 135150) is an autosomal dominant disorder characterized by benign hair follicle tumors, pneumothorax and kidney malignancies. It is caused by nonsense mutations and deletions affecting the *BHD* gene encoding for folliculin (FLCN). FLCN is thought to be a negative regulator of mTOR (mammalian Target Of Rapamycin), a central player in cellular responses to growth signals. Two binding partners (FNIP1 and FNIP2) are known. FNIP1 may interact with AMPK, a kinase upstream of mTOR. Not much is known about FLCN's binding partners or manner of interaction with the mTOR network. FLCN is supposed to be a cytoplasmic protein, but it is known that many proteins shuttle between cellular compartments as part of their normal function. In order to more fully understand its function, we decided to study FLCN's subcellular localization in more detail because it might be possible that mutations affect FLCN's localization and, in that way, its function.

To examine how FLCN's subcellular localization is influenced by mutations and/or by culture conditions that influence mTOR signalling we have prepared N-terminal EGFP-tagged FLCN expression construct (CMV promotor). Using site directed mutagenesis, we introduced several known mutations into the constructs. These constructs and a wild type were transfected into HEK293 cells and followed by fluorescence microscopy. Next, we repeated the experiments under conditions known to influence mTOR signaling: hypoxia, starvation and rapamycin administration to see if mutations affect FLCN's response to these alterations in the environment. Results from these experiments will be presented.

**P06.009****Evaluating the expression of BMI1 gene and protein in bladder tumors by means of RT-PCR and immunohistochemistry****M. Malekzadeh Shafaroudi<sup>1</sup>, A. Malekzadeh<sup>2</sup>, S. J. Mowla<sup>2</sup>, A. R. Bahrami<sup>3</sup>**<sup>1</sup>Sari Medical Faculty, Sari, Islamic Republic of Iran, <sup>2</sup>Tarbiat Modares University, Tehran, Islamic Republic of Iran, <sup>3</sup>Institute of Biotechnology, Ferdowsi Uni-

versity of Mashhad, Mashhad, Islamic Republic of Iran.

BMI1, a Polycomb group repressor protein, represses the genes that induce cellular senescence and cell death, and it can contribute to cancer when improperly expressed. We aimed to evaluate the expression and tissue distribution of BMI1 in bladder tumors. Tissue specimens containing bladder tumor were evaluated and compared with intact tissues from tumor margins and normal bladders. Tumor specimens of patients with transitional cell carcinoma of the bladder, and tumor-free tissues taken from the margin of the tumors were obtained and tested for the expression of BMI1 gene and protein. Specific primers for BMI1 and B2M (as an internal control) were used for reverse transcript polymerase chain reaction technique. The production and distribution of BMI1 protein was also examined by immunohistochemistry and western blotting techniques. Polymerase chain reaction generated a 683-bp product, corresponding to the expected size of BMI1 amplified region. The identity of the amplified fragment was then confirmed by direct DNA sequencing. The mean of expression of BMI1 detected in tumor tissues was significantly higher than that in intact tissues, and there was also a significant association between the mean of gene expression and the stage of malignancy ( $P < .001$ ). The expression of BMI1 at protein level was further confirmed by western blotting and immunohistochemistry. BMI1 is a potent repressor of retinoblastoma and p53 pathways, and hence, elucidating its role in tumorigenesis is very important. We reported for the first time the expression of BMI1 and its correlation with incidence and progress of bladder tumors.

#### P06.010

##### The role of the *CYP1A1*, *GSTM1*, *GSTP1* gene polymorphisms in formation of predisposition to bladder cancer

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Bladder cancer is a wide spread oncological disease with numerous risk factors. Such factors include carcinogens of tobacco smoke, polycyclic aromatic hydrocarbons and other substances, collectively named xenobiotics. Xenobiotics become active in hypersensitive organisms, sensitivity of which is formed under a specific genetic background. That is the reason for the importance of studying peculiarities of genetic systems, which take part in detoxication of xenobiotics.

To detect associations of polymorphisms of the *P450* cytochrome (*CYP1A1*(*lle462Val*)) and glutathione-S-transferases (*GSTM1*(*del*), *GSTP1*(*lle105Val*)) genes in formation of predisposition to bladder cancer we genotyped 145 patients (77 patients had surface form, and 68 had invasive form of this disease) and 241 controls by PCR-RFLP analysis. All individuals were citizens of the Republic of Bashkortostan selected on the basis of age, sex and ethnicity.

That genotypes *lle/Val* of *CYP1A1* gene ( $OR=8.6$ ,  $p=0.0005$ ), *Val/Val* of *CYP1A1* we found gene ( $OR=7.8$ ,  $p=0.006$ ), *Val/Val* of *GSTP1* gene ( $OR=3.3$ ,  $p=0.01$ ) were significantly associated with bladder cancer. A positive association were found between a *CYP1A1* *Val/Val* genotype, a *GSTP1* *Val/Val* genotype and urinary bladder carcinoma invasive forms ( $OR=10.9$ ,  $p=0.01$  и  $5.2$ ,  $p=0.01$  respectively).

There were no differences in *GSTM1* gene deletion frequency distribution groups in both.

The data shown that polymorphisms of the *P450* cytochrome and glutathione-S-transferases can be important components of the genetic structure of predisposition to the development of cancerous neoplasms.

#### P06.011

##### Investigation of Cytochrome P-450 Polymorphisms in Turkish Bladder Cancer Patients

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Bladder cancer is the fourth most common cancer in men and the seventh in women globally. Well-known etiological risk factors are occupational exposure to certain carcinogens and cigarette smoking. It is thought that the interaction between genetic factors and the environment accounts for the different levels of susceptibilities to the development of bladder cancer.

The cytochrome P-450 (CYP) superfamily of enzymes catalyses one of the first steps in the metabolism of carcinogens such as polycyclic aromatic hydrocarbons, nitroaromatic compounds and arylamines. This

enzyme system is highly polymorphic and these polymorphisms within CYP genes have been found to be associated with many cancer types. In addition, frequency of CYP polymorphisms shows ethnic variations among populations, so it necessitates population based studies.

In this study, *CYP1A1* (m1, m2, m3, m4), *CYP1B1* (Leu432Val), *CYP2D6* (int3/ex4) and *CYP2E1* (PstI, RsaI, DraI) polymorphisms and their interaction with environmental factors such as sex, age and smoking are studied in Turkish population and association between these polymorphisms and bladder cancer is investigated. Investigated *CYP1A1*, *CYP2D6* and *CYP2E1* genes are shown not to be associated with the disease, however, it is found that *CYP1B1* 432Val allele increases the risk of bladder cancer (adjusted OR = 3,080, 95% CI: 1,204 - 7,874). Age, sex and smoking are also found to be associated with increased risk of bladder cancer.

#### P06.012

##### Gene expression in superficial bladder cancer using microarrays

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Non-muscle invasive bladder cancer is a heterogenous disease whose molecular phenotypes are being elucidated. Treatment of these superficial tumours is dependent on the risk of recurrence. To improve the accuracy of progression prediction various molecular markers have been evaluated by gene expression microarrays but to date no molecular markers have been used in clinical practice. For the improvement of recurrence prognosis we analysed gene expression and identified differences between superficial bladder tumours in a non-recurrence cohort during period of two years (19 patients) and early recurrence cohort (18 patients), which might explain differences in the biology and clinical outcomes. Data from microarrays containing 29,019 targets (Applied Biosystems) were subjected to a panel of statistical analyses to identify bladder cancer recurrence-associated gene signatures. Initial screening using the GeneSpring and Bioconductor software tools revealed a putative set of about 120 genes associating with the early recurrence cohort. Significant differences were observed by *CDKN2A*, *CDKN1C*, *HOXA10*, *GPNMB*, *TCN1*, *INA*, *H19*, *AURKC*, *FABP3* and *PLD2* genes. Besides, we integrated the microarray dataset with additional background knowledge, in order to algorithmically mine for differential-expression patterns in terms of the Gene Ontology functions and processes as well as known regulatory pathway memberships. Our results indicate that it may be possible to identify patients with a high risk of disease recurrence at an early stage using a molecular profile present already in the superficial tumours. Research was supported by MSM 0021620808.

#### P06.013

##### Investigation of the relationship between mitochondrial DNA and transitional cell carcinoma of the bladder

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Mitochondrial DNA mutations have been described recently in different tumors, whereas similar studies focusing on bladder cancer are scarce. In an effort to understand the significance of mtDNA mutations in bladder cancer, we investigated the mtDNA alterations. It has been suggested that the extent of mitochondrial DNA mutations might be useful in the prognosis of cancer and the response to certain therapies.

In an attempt to progress in the understanding of the relationship of mtDNA alterations and bladder tumorigenesis, we studied the mtDNA in 38 bladder tumors and 21 microdissected normal bladder tissues. Mitochondrial genes ATPase6, Cytb, ND1 and D-loop region were amplified by PCR. The results of DNA sequencing revealed numerous point mutations in mtDNA genes. We detected 40 mutations in the pa-

tient group in which 19 of them resulted in aa substitution. Our findings indicate that G8697A, G14905A, C15452A and A15607G mutations are frequent in bladder cancers ( $P < 0.05$ ). These mutations are belong to mtDNA haplogroup T. No association was found between mtDNA mutations and the patients' gender, tumor stage or grade, recurrence and progression.

In conclusion, the high incidence of mtDNA mutations in bladder cancer suggests that mtDNA and mitochondria could play an important role in the process of carcinogenesis process. Especially, mtDNA haplogroup T may be important to detect the cancer risk. More extensive biochemical and molecular studies and further studies in larger groups are necessary to determine the pathological significance of these mutations.

#### P06.014

##### Molecular genetic alterations and their predictive values in superficial bladder cancer.

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Conventional histopathologic and morphologic factors are widely used to predict poor prognosis in patients with superficial bladder cancer (BC) underwent transurethral resection. But this system is not able accurately predict the behavior of the most bladder tumors and need additional factors.

Our purpose was to establish associations between some genetic alterations in respect to unfavorable clinical phenotype (recurrence rate, invasion, high grade) and to determine the prognostic significance of these genetic alterations.

We have studied 108 matched samples (blood and tumor tissue) from patients with superficial BC, of which 12 patients demonstrated recurrence within one year, and 10 samples from patients with invasive BC. The panel included loss of heterozygosity at 3p14, 9p21, 9q34, p53 locus, activating mutation in 7 exon FGFR3 and RASSF1A, p16, p14, RAR $\beta$ , CDH1 promoter hypermethylation. Methods: microsatellite analysis, SSCP and direct sequencing and methyl-sensitive PCR. Statistical analysis included comparison of the patients' clinical groups by Fisher's exact test, calculation of odds ratios and corresponding 95% confidence intervals.

Results: 9p21- locus deletions are significantly more frequent in primary tumors with high recurrence rate (within one year) ( $p=0.049$ . OR=8.70). FGFR3 mutations are associated with Ta stage ( $p=0.0042$ . OR=5.00). 3p14 locus deletions ( $p=0.042$ . OR=5.71), RAR $\beta$  ( $p=0.016$ . OR=3.91) and p16 ( $p=0.055$ . OR=4.17) promoter hypermethylation are associated with high grade tumors. P53 locus deletions ( $p=0.006$ . OR=8.10) and p16 hypermethylation ( $p=0.05$ . OR=4.09) are significantly more frequent in invasive bladder tumors than in superficial tumors. Conclusion. Revealed genetic alterations could be used as additional prognostic markers to predict tumor's behavior more accurately.

#### P06.015

##### Diagnostic and prognostic molecular markers in brain tumors

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Malignant gliomas are associated with high morbidity and mortality. Despite optimal treatment, the survival is often very low. Recently, there have been advances in our understanding of the molecular pathogenesis of gliomas and progress in treating them. This work summarizes preliminary results of molecular markers routinely used to help diagnosis and management of these tumours in adults. Loss of heterozygosity (LOH) on 1p and 19q seem to be a predictor of good prognosis and chemosensitivity. Conversely, 10q LOH predicts rather poor outcome. Moreover, epigenetic silencing of the MGMT promoter predicts response to alkylating agents.

We have analyzed 32 cases: 18 glioblastomas, 10 oligodendrocytomas and 4 astrocytomas. After DNA extraction from peripheral blood and

microdissected-FFPE samples, we analyzed by microsatellite LOH on 1p, 19q and 10q and the MGMT promoter by methylation-sensitive PCR. Two cases (1 astrocytoma and 1 glioblastoma) were not analyzable due to low quality of tumor DNA.

All astrocytomas did not show LOH on chromosomes 1p, 19q and 10q. Among the 17 glioblastomas, two did not show LOH, three showed 1p LOH, two 1p+19q LOH, one 1p+10q LOH, one 19q+10q LOH and eight 10q LOH. All oligodendroglomas exhibited 1p+19q LOH. MGMT promoter resulted methylated in all oligodendroglomas and astrocytomas, while among glioblastomas only 6 were methylated. As reported in literature, 1p and 19q LOH are hallmarks of oligodendroglomas, while LOH do not seem to be associated to astrocytomas. These results confirm the correlation between histological diagnosis and genetic molecular markers; follow up on treatment response is underway.

#### P06.016

##### Polymorphisms in DNA BER genes XRCC1 and PARP1 increase the risk of adult brain tumors

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Cancer development is a multi-step process, in which polygenic and environmental factors play important roles. Allelic polymorphisms of genes that code for DNA repair enzymes could also determine genetic susceptibility or resistance to cancer in most instances. XRCC1 and PARP1 are two important proteins in base excision repair. In this hospital-based case-control study, consisted of 137 patients with primary brain tumors and 244 cancer-free control subjects, we sought to determine possible associations between XRCC1 Arg399Gln and PARP1 Val762Ala polymorphisms and primary brain tumors. We also searched for any possible relation between the tumor grade and these polymorphisms. The results indicated a significant association between the occurrence of primary brain tumor and the existence of XRCC1 Arg399Gln and PARP1 Val762Ala polymorphisms (OR: 2,113; 95% CI: 1,069-4,176;  $p=0.041$  and OR: 3,720; 95% CI: 1,673-8,273;  $p=0.001$ , respectively). However, there was no statistically significant relationship between the tumor grade and the polymorphisms studied. We conclude that XRCC1 Arg399Gln and PARP1 Val762Ala polymorphisms might play role in the initial stages but have no effect on the progression of brain tumor development.

#### P06.017

##### Cancer genes mutation testing - an improved sequencing workflow for quality-assured, clinical-grade results using improved technology capillary electrophoresis

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It was found that the range of mutations that can drive cancer growth could be much wider than initially expected. An international research effort, the Cancer Genome Project, has identified around 120 genes that may contain mutations promoting the disease. Moreover, in the recent years it has been demonstrated that cancer gene mutations can not only report about cancer predisposition like in BRCA1 and BRCA2 genes but that they can also serve as biomarkers for therapy decisions and so in clinical practice mutations in the genes KRAS, EGFR and more recently BRAF are being used for that purpose. This has brought cancer gene mutation analysis from a research study to a clinical management practice. For this reason also the needs for the technology capable of performing genetic analysis changed from high productivity features to an easy to use technology. Here we describe the results of independent collaborative studies to develop improved sequencing workflows on several selected cancer genes. These protocols take advantage of new reagents for better sensitivity and the latest generation capillary electrophoresis instruments, easy to use and with new level of data quality. Quality control features built in this new technology will offer new solutions to the clinical laboratory for obtaining results with the highest level of reliability and quality.

**P06.018****Expression of Fibroblast Growth Factor Receptor-1 in tumors of mexican females**

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Expression of Fibroblast Growth Factor Receptor-1 in tumors of Mexican females.

In Mexico, breast cancer is the second leading cause of cancer death in women 30 to 54 years and ranks as the leading cause of mortality from malignant neoplasms among women. Fibroblast growth factors are a family of ligands that signal through receptor tyrosine kinase, has been linked to processes such as proliferation, differentiation, cell migration, angiogenesis and its inappropriate expression has been implicated in breast cancer. It is believed that these receptors are linked to the progression to the independence of steroids in breast cancer. It has been shown that the expression of FGFR1 is essential in mammary development and breast cancer, and the FGFR1 gene shows increased levels of mRNA and proteins. Therefore, the aim of this study is to assess the level of FGFR-1 expression in mammary tumors from Mexican women by RT- PCR and Real Time RT-PCR . The tissues were obtained from the Hospital de Oncología CMN of S-XXI. 15 tumors were used in sporadic stage II and III breast cancer, 5 unaffected breast tissues and cultured cell lines MCF-7, MDA-MB-231, MCF10A as a positive control. First we found that cell lines of breast cancer expressed higher levels of FGFR-1 than the cell line of normal mammary epithelium. Similarly, the tumors expressed higher levels of FGFR-1 than unaffected breast tissue. We conclude that FGFR-1 is associated with disease progression and can be used as a tool for diagnosis, prognosis and treatment of breast cancer.

**P06.019****Characterization of an Oncogene Candidate, USP32, on 17q23**

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Breast cancer is one of the most common malignancies affecting women. The lifetime risk of any woman getting breast cancer is about 1 in 8. Gene amplification is a common mechanism of oncogene activation in cancers. Previous studies identified the chromosomal band 17q23 as a frequent site of gene amplification in breast cancer. Amplification and overexpression of oncogene candidates in this region are to be likely important for breast cancer. USP32, a predicted ubiquitin specific protease on 17q23, is one of the oncogene candidates. We hypothesized that overexpression of USP32 may contribute to breast tumorigenesis as a protein taking part in protein degradation pathways. We detected amplification and overexpression of USP32 in MCF7 and BT474 breast cancer cell lines. Presence of Cys-His domain suggests that USP32 functions as a deubiquitinating enzyme. To experimentally confirm this, we sub-cloned three different fragments containing functional domains of USP32 into a GST fusion vector. These constructs were tested in vivo for deubiquitination activity and we demonstrated that this protein is indeed a ubiquitin specific protease. To understand the physiological role of this protein inside mammalian cells, we performed localization and co-localization experiments for full length and partial fragments of USP32 by generating USP32-GFP fusions. For further localization studies, we used Fluorescent Protease Protection assay which suggested that USP32 could be a membrane bound protein. Further characterization of USP32 will help us understand the role of this protein in the cell and in mammary tumorigenesis.

**P06.020****Integration of the Fluidigm technology into a high throughput genotyping laboratory**

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The Centre for Genetic Epidemiology is using high-throughput SNP genotyping to identify and verify genetic variants that underlie susceptibility to various cancers. Cancers that are being investigated in-

clude breast, ovarian, colorectal, prostate, and melanoma. The Centre has recently integrated the Fluidigm® technology into its core, high throughput, genotyping laboratory.

Starting with an initial pilot experiment to demonstrate accurate SNP calling, 384 DNA samples were genotyped using 48 TaqMan® assays. These genotypes were compared with those previously obtained using conventional genotyping with the same TaqMan assay. Further work confirmed that the Fluidigm EP1 system meets high throughput needs, which can be further scaled up easily, when needed, and provides a fast and efficient workflow. The overall process flow was configured so as to handle large sample numbers, including automated liquid handling with associated sample tracking and data QC. The first production experiment, using the 96.96 dynamic arrays and the EP1 instrument, genotyped 96 SNP assays across 1200 endometrial cancer samples, generating over 115,000 genotypes. The Centre plans to use the system in genotyping studies involving thousands of samples, studies that were previously less feasible due to workflow and cost constraints.

**P06.021****Expression and prognostic significance of cell cycle regulatory genes CDKN1A, TP53, CDK4, CDKN2 in breast and colon cancer**

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Aim of this study was detection of alterations in expression of cell cycle control genes: CDKN1A TP53, CDK4 and CDKN2 in breast and colon cancer patients.

CDKN1A encodes protein p21 which binds to and inhibits the activity of CDK4 complex and thus regulates transition of cell through G1 phase of the cell cycle. CDKN1A gene is under direct control of tumor suppressor gene TP53 which induces G1 phase arrest in response to a variety of stress. Mutations may destroy the normal function of p53 as a transcription factor and induction of cell arrest or apoptosis may be reduced. This study included 100 breast and colon cancer affected individuals. Genetic material was extracted from biopsic tissue specimen. To analyze effects of mutations on expression of tested genes, TP53 was subjected to mutational analysis using RFLP. Level of expression of CDKN1A, TP53, CDK4 and CDKN2 was determined using SYBR-green based real time PCR.

All relative quantity values of TP53 and CDK4 were normalized to mRNA level of beta-actin and their relative expression was calculated using REST®software.

To test hypothesis that alteration in sequence and expressions of tested genes may have a prognostic significance, experimentally obtained data were correlated to pathohistological findings.

**P06.022****Investigation of the mitochondrial DNA deletions in patients with chronic cervicitis and cervical cancer and of the correlations between mtDNA deletion and pathological diagnosis**

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Mitochondrial DNA (mtDNA) is the specific DNA molecule within mitochondria and susceptible to mutations because of a disability of DNA repair mechanisms and more exposure to oxidative stress. The aim of the present study was to investigate the mitochondrial DNA deletions in the tissue of cervix uteri of the patients with chronic cervicitis and cervical cancer and to explain the correlations between mtDNA deletion and pathological diagnosis.

Tissue samples of cervix uteri were collected from paraffin blocks of 60 individuals in pathology archives; 20 patients with chronic cervicitis (ChC group), 20 patients with cancer of cervix uteri (CaC group) and 20 patients, as control group, undergo an operation of uterotomy because of other disorders. After mtDNA extraction, PCRs were made using three different primer pairs; first primer pairs for a deletion of 4977 bp, the second primer pairs for negative control within the deletion region and the third primer pairs for positive control out of the deletion region.

We did not detect a deletion cervix uteri tissue of control group. However, we determined a heteroplasmic 4977-bp deletion in ChC group. The deletions were homoplasmic in CaC group.

It was suggested that overproduction of reactive oxygen species in

chronic inflammation may cause oxidative damage of mtDNA. In addition, we suggest that mtDNA deletions may be predisposing or accelerator factor on malign transformation of cells of cervix uteri.

#### P06.023

#### Nucleostemin is highly expressed in gastric adenocarcinoma and its gene expression knock-down caused a G1 arrest in AGS cell line

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Nucleostemin (NS) is a nucleolar protein, primarily hypothesized to be a novel regulator of stem cell self-renewal, where it binds to P53 and hence regulates cell cycle. It is now believed that NS is also expressed in some highly proliferating cells of adult tissues including several cancer cell lines and tissues. However, molecular links between NS and stem-cell self-renewal, embryogenesis and/or tumorigenesis are not well understood. We have previously shown that NS is highly expressed in rat bone marrow stromal stem cells (BMSCs) and the expression is shut off upon induction of differentiation. Knocking-down the expression of NS in rBMSCs suggests an apparently P53-independent role for it in these cells. Moreover, NS knock-down in different bladder cancer cell lines demonstrated a cell-type dependent function of NS in arresting cell proliferation in either G1 or G2/M phases of cell-cycle. Here, we have evaluated the potential expression of NS in 40 tumor/non-tumor biopsies of the patients with gastric cancer. Moreover, we have assessed the effects of the NS knock-down on cell proliferation of the AGS cell line, using RNAi strategy. Our data revealed that NS is highly expressed in gastric adenocarcinoma, but not in apparently normal tissues obtained from the margin of same patients. The data suggest that NS is potentially a suitable tumor marker for diagnosis, grading and molecular classification of gastric adenocarcinoma. Furthermore, NS knock-down in the AGS cell line caused a G1 cell cycle arrest in the cells, confirming its causative role in gastric tumorigenesis.

#### P06.024

#### Targeting Cancer stem cells via OCT4 promoter-derived construct

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Despite significant improvements in cancer therapy, tumor recurrence is frequent and can be due to a variety of mechanisms, including the evolution of resistance and tumor metastasis. Cancer stem cells are a subclass of cancer cells possessing parts of properties of normal stem cells. The existence of these malignant stem cells has been proven for hematological as well as some solid tumors. These kinds of cells have a high capacity of proliferation and are not targeted by standard therapy thus play a critical role in tumor recurrence, resistance to radio- and chemotherapy. The Octamer-binding transcription factor 4 gene (OCT4), encodes a POU-domain transcription factor which plays a critical role in maintaining pluripotency and self-renewal of ES cells. OCT4 expression has also been detected in adult stem cells as well as a variety of cancer cells and cell lines e.g. the bladder 5637 cell line. In this study, the OCT-4 promoter was cloned into the pGL3-control reporter vector encoding the Luciferase gene, followed by transfection of the resultant construct into the 5637 bladder cell line. OCT-4 promoter-driven Luciferase gene expression was further detected in the 5637 bladder cell line which suggests the activity of the OCT-4 promoter in these cells. Our data indicate that OCT4 can be chosen as a therapeutic target, as well as a novel tumor biological and prognostic marker which could decrease the toxicity of cancer therapy to normal tissues.

#### P06.025

#### Expression of DDX3Y in testicular germ cell tumours (TGCTs) - a model system for the study of DDX3Y male germ line function

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DDX3Y is a DEAD-box-RNA-helicase located on the Y-chromosome. It has a functional counterpart on the X-chromosome, DDX3X, and both are involved in translational control of downstream genes and control of G1/S-progression during cell-cycle. While DDX3Y protein is exclusively expressed in the male germ-line, predominantly in spermatogonia, the DDX3X protein is found also in somatic cells, but in the male germ-line only translated in spermatids. Since the deletion of DDX3Y results in pre-meiotic spermatogenic disruption, an essential role of DDX3Y in spermatogenesis is assumed. Our purpose was to examine if the mechanisms for translational regulation of DDX3Y transcripts might be associated with male germ-cell differentiation. Testicular germ-cell tumours (TGCTs) are derived from different stages of germ-cell maturation and therefore provide a model-system for such germ-cell-differentiation studies. Consequently, we used a TGCT sample panel including the pre-invasive carcinoma *in situ* (CIS) to investigate the expression of DDX3Y protein in these specimens. Our results revealed strong DDX3Y expression in CIS cells but a heterogeneous pattern in other TGCT cell types. The strong CIS expression is marking their high proliferative activity and clearly designates DDX3Y as a novel marker for these tumour precursor cells. Overt tumours showed only a small and variable number of DDX3Y expressing cells. An abundant expression in seminomas compared to non-seminomas points to reduction of DDX3Y expression during tumour-progression. Our results indicate a successive vanishing of the spermatogonia cell type character of these tumour cells and suggest that the spermatogonia specific DDX3Y translation is indeed controlled by germ-cell specific trans-factors.

#### P06.026

#### From human papillomaviruses & cervical carcinoma to HPV detection

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Human papillomaviruses (HPV) are double-stranded DNA viruses, invading mucosal or cutaneous epithelium. More than 100 different types of HPV are already known.

With respect to the oncogenic potential, HPV are divided into "high-risk"(HR) and "low-risk"(LR) types. HR HPV invade ano-genital and oropharyngeal regions of the human body and cause one of the most serious and the second most frequent cancer in women: cervical carcinoma. HPV DNA carries information for proteins of an early (E-proteins) and a late (L-proteins) infection phase. Most serious is the interaction of E6- and E7-proteins with the human p53 and pRb proteins, respectively, leading to malignant transformation of infected cells. Within this presentation we would like to draw your attention to relations between cervical carcinoma, HPV infection and detection. HPV detection can be performed by different techniques: indirect detection, showing us presence of altered tissues or cells. The most widely used technique, but not the most sensitive one, is gynaecological cytology. Direct detection, manifesting presence of HPV, uses (among others) most sensitive molecular biology techniques, and makes HPV DNA testing possible. We have tested 380 patients. 88 patients (23.1 %) were found to be HPV DNA positive.

Use of the HPV DNA test allows detection of HPV infection with high sensitivity and specificity. Such result can more efficiently specify unclear outcomes of other screening methods, such as cytology. HPV DNA test has been already incorporated into routine screening within several countries. This test can be used for pre-vaccination (e.g. before vaccination against HPV) examination too.

#### P06.027

#### Genome-wide discovery of copy number variations in cancer patients using SOLiD sequencing

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Copy number variations (CNVs) have been widely observed in tumor genomes and recognized as one of the causes of cancer progression. Massively parallel sequencing provides a powerful, accurate and unbiased way to interrogate CNVs across the whole genome. We sequenced matched pairs of tumor and normal samples of three patients with tongue/tonsillar cancer using the SOLiD™ System, and we report genome-wide discovery of CNVs. Using a modified version of the Seg-Seq algorithm and controlling the false discovery rate, we compared the numbers of sequence reads from tumor samples to those from normal samples in 100kb windows; we identified at least 300 significant copy number changes (gains and losses) per genome, with a maximum observed copy number of 9x. The size range of CNVs was 1 kb to 71,000 kb. In parallel, we developed a novel pipeline for identifying CNVs using only a single sample.

Using a new total RNA-based protocol, we SOLiD-sequenced the whole transcriptome of the tumor and normal samples, and examined the correlation between copy number variation and changes in gene expression. We found a significantly positive correlation (0.56) between CNV and gene expression in a patient. Some genomic segments with big increases in copy numbers in genomic tumor samples also show significantly elevated expression levels in the tumor transcriptome compared to the normal transcriptome. It suggests that the structural mutations in tumors may drive changes in gene expression. The identified CNV segments offer insight into genes associated with the initiation or progression of cancer.

#### P06.028

#### Role of *PTEN* gene in the development of prostate cancer: should male patients affected with Cowden syndrome be screened for prostate cancer?

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**Introduction:** *PTEN* (locus 10q23.31) is a tumour suppressor gene encoding a dual phosphatase protein that negatively regulates the PI3K/Akt/mTOR pathway. Somatic loss of *PTEN* has been described in sporadic human cancers: brain, bladder, colon, lung, breast, endometrium and prostate. Germline mutations of *PTEN* cause a spectrum of autosomal dominant hamartomatous overgrowth disorders including Cowden syndrome (CS, #158350). CS is mainly characterized by macrocephaly, facial trichilemmomas, acral keratoses, papillomatous papules and increased risk for breast, thyroid and endometrial cancer. Surveillance for susceptible malignancies is the mainstay of management these patients.

**Clinical Report:** We report on a 58 years old male patient affected with CS. He presented macrocephaly, sclerotic fibroma, meningocele, gastrointestinal polyps and renal tumour. At the age of 56 he was diagnosed a high grade prostate cancer, already metastasized (vertebral column). Mutation analysis of *PTEN* in DNA extracted from lymphocytes allowed the detection of a heterozygous mutation in exon 5 of *PTEN* gene c.388C>T (p.R130X).

**Discussion:** Although CS is a hereditary syndrome of cancer susceptibility, guidelines of surveillance of this disease don't comprise screening for prostate cancer. However, there's compelling evidence indicating a role for *PTEN* in the initiation and progression of prostate cancer (due to loss of heterozygosity and/or cooperation with other genes). This report highlights the importance of performing large scale studies in patients with CS to evaluate the presence of (pre)neoplastic lesions of the prostate, which might be significant. The confirmation of this hypothesis would validate the inclusion of prostate cancer screening in the surveillance protocol of CS.

#### P06.029

#### Cyclooxygenase-2 gene polymorphism and prostate cancer

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kara, Turkey, <sup>2</sup>Ankara University, Faculty of Medicine, Department of Urology, Ankara, Turkey.

**Aim:** Cyclooxygenases (COX) play role in biosynthesis prostaglandins from arachidonic acid. There are two COX isoforms, COX-1 and COX-2. COX-2 expression is very low under physiological conditions in most organs, but it can be induced. The over-expression of COX-2 was observed in many cancers associated with reduced apoptosis and promoting of angiogenesis. The polymorphisms in which is determined in COX-2 gene change gene expression. The COX-2 expression changes in 765G→C polymorphism. Our aim was to determine the allelic frequencies of the -765G > C COX-2 polymorphism in prostate cancer.

**Methods:** 29 prostate cancer patients and 40 healthy controls were in this study. Genotyping were carried out with PCR-RFLP in extracted DNA.

**Results:** In control group, the frequency of persons carrying COX-2 GG, GC and CC genotypes were 57.5%, 37.5%, 5%, respectively. In patient group, the frequencies were 72.4% GG, 20.7% GC, 6.9% CC. There was no a significant difference between groups in terms of the distribution of COX-2 genotypes and alleles ( $p>0.05$ ).

**Discussion:** Altered COX-2 gene expression effects invasiveness of malignant cells, the rate of apoptosis and angiogenesis. COX-2 GC or CC genotypes reduces gene expression. Therefore, we expect that the frequency of GG genotype is more than CC genotype in cancer. Our data support this hypothesis. Although there is not a significant difference in prostate cancer, we found a tendency of GG genotype rather than control group. For our knowledge this is the first study to analyze the association between COX-2 gene polymorphism and prostate cancer in Turkish population.

#### P06.030

#### Initiation and progression of Prostate cancer in cohort of North Indian population by cytokine gene polymorphisms

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**Background:** Chronic intraprostatic inflammation is suspected to play a role in the pathogenesis of prostate cancer (PCa). Polymorphisms in cytokine genes influences PCa development via regulation of the anti-tumor immune response and/or pathways of tumor angiogenesis. Thus, the present study was undertaken to evaluate the association of cytokine gene polymorphisms for the susceptibility to PCa

**Materials and methods:** Our study included 197 histologically confirmed cases of adenocarcinoma of prostate and 256 healthy controls of similar age and ethnicity. *IL-1 RN* (intron 2 VNTR), *IL-1 B* -511 C>T, *IL-4* (intron 3, VNTR), *IL-6* (-174 G>C), *IL-10* (-819 C>T and -1082 G>A), *TNF-A* (-1031 T>C, -863 C>A, -857 C>T, -308 G>A) and *IFN-G* (+874 A>T) genes were genotyped by PCR-RFLP or ARMS-PCR based method

**Results:** Our results illustrated that gene variant of *IL-10* -1082 and *TNF-A* -1031 to be associated with two fold increased susceptibility for PCa. Our results also demonstrated that variant genotypes of *IL-6* -174 G>C (OR-2.07,  $p=0.012$ ) and *TNF-A* -1031 T>C (OR-2.14,  $p=0.022$ ) polymorphisms to be associated with progression to bone metastasis in PCa patients. Moreover *IL-10* haplotype T(-819)-G(-1082) demonstrated 70% decrease in risk for PCa progression.

**Conclusion:** The observations from this exploratory study suggested that polymorphism in cytokine genes (*IL-10*, *TNF-A*, and *IL-6*) to be associated with PCa risk and may have a significant effect on prostate cancer development and progression. However, a definitive study of SNPs in large cohort and different ethnicity influencing this pathway to investigate possible associations with prognosis is needed.

#### P06.031

#### Analysis of frequency *CHEK2*, *P53*, *NOD2/CARD15* and *RET* gene polymorphisms in polish patients with differentiated thyroid cancer

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Thyroid carcinomas are the most often carcinomas of endocrine system with still growing up frequency. The most often occurs papillary and fol-

icular thyroid cancer (80-90%), which belong to group of tumors well prognoses and slowly progress and benignity. Very serious problem are recurrences and regional or remote metastasis. Progression from well differentiated thyroid cancer to malignant anaplastic carcinoma is possible also. In this focus, very important seems to be searching for molecular markers of disease course, good or poor prognosis and response on medical treatment as well. It is expected that polymorphisms research in genes demonstrating association with neoplastic diseases will be helpful in understanding of molecular mechanisms of thyroid gland tumors development. The dependence of differential thyroid cancer occurrence on DNA variation: I157T in CHEK2 gene, R72P in P53 gene, 1007fs in NOD2/CARD15 gene and synonymous G2497T substitution in RET protooncogene was examined. 296 patients with differentiated thyroid cancer and 200 individuals from population group was examined. We used pyrosequencing technique. There were no significant differences in allele or genotype frequencies in analysis of RET G2497T substitution and R72P in P53 gene but mutated allele frequencies of 1007fs and I157T was 8,95% and 4,9% in patients with thyroid cancer, compared with 2,92% and 2,1% in control individuals respectively. Our findings indicates that particular characteristics of cancer risk genes on RNA level as well as DNA changes is necessary. Additionally a summary effect of different SNP changes as a cancer predisposing factor is possible, so further analysis will be performed.

#### P06.032

#### Two cases of double heterozygotes in families with hereditary cancer

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BRCA1 and BRCA2 genes are tested in all familial or sporadic early onset breast and ovarian cases, which fulfil inclusion criteria. Two of the CHEK2 gene mutations, c.1100delC and deletion of exon 9-10, are also tested as a last step of examination, in all BRCA1/2 negative probands. MLH1, MSH2 and MSH6 genes are tested in cases of possible HNPCC syndrome. In one of our families both BRCA1 deletion of exon 20 and CHEK2 c.1100delC was detected in a proband with breast cancer at 44; her mother had breast cancer at 40 and ovarian cancer at 48. Proband has three daughters, one non-carrier, one carrier of CHEK2 mutation and one carrier of BRCA1 deletion. In other family originally tested for Lynch syndrome, MLH1 mutation (splice site) was detected in a proband with rectal cancer diagnosed at 58, whose father and grandfather had stomach cancer. Because his sister had breast cancer and mother had ovarian cancer, additional testing was provided and a BRCA1 mutation (protein truncating) was discovered. The occurrence of double heterozygotes is possible but probably rare. The clinical geneticist should carefully evaluate family history and indicate testing of additional hereditary cancer syndromes especially in probands with positive cancer occurrence from both parents. The preventive recommendations should be explained even in non-carriers of high-risk familial mutation. *Supported by the Ministry of Health of the Czech Republic: Grant MZOMZO2005*

#### P06.033

#### Novel variants of fusion gene transcripts in soft-tissue sarcomas.

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Soft-tissue sarcomas have specific recurrent chromosomal translocations producing chimeric gene fusions, which regarded as a major factor in the development of these tumors. Fusion of FUS and CHOP gene is a common genetic event found in liposarcomas. Majority of Ewing's sarcomas are associated with fusion gene EWS-Fli1. These specific translocations can be used for exact diagnosis in poorly differentiated tumors.

We examined 28 formalin-fixed paraffin-embedded sarcoma samples (20 liposarcomas, 8 Ewing's tumors). Translocations were tested by FISH, characterization of fusion genes FUS/CHOP, EWS/FLI1 was performed by RT-PCR and sequencing.

We observed translocations in 18/20 cases of liposarcomas and in

8/9 cases of Ewing's tumors. In 19/25 cases endogenous control B2M indicated adequate RNA quality. We found EWS/FLI1 fusion genes in 3/8 Ewing's sarcomas. In 2 cases ews exon 7 being fused to fli1 exon 6, in 1 case ews exon 7 being juxtaposed to 10 ews exon following by fli1 exon 6 (novel fusion variant).

We detected FUS/CHOP fusion gene in 45% (5/11). All cases had a type fusion transcript involving fusion of FUS exon 5 with CHOP exon 2. Two novel transcript modifications were found, of which one had 6 nucleotides insertion and the other lacked 14 nucleotides of CHOP. Moreover we found two different variants of the transcript in one sample, caused by alternative splicing apparently.

Further investigation of structural attributes of fusion genes may be helpful for the prospective target therapy of patients with such fusion oncogenes and give insight into the malignant sarcoma development.

#### P06.034

#### Different genetic reasons of gastric cancer

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Mutations of CDH1 gene are associated with familial diffuse gastric cancer in some populations. MLH1 and MSH2 mutations also may predispose to gastric cancer.

Three samples of patients for mutations and SNPs in the CDH1, MLH1 and MSH2 genes were studied: 30 patients with familial gastric cancer, 99 patients with sporadic gastric cancer and a control sample of 112 probands by SSCP and sequencing.

Mutations in gene CDH1 have not been found. We investigated our sample of familial gastric cancer and found 5 germline MLH1 and MSH2 mutations (16,6%). We have studied the significance of the CDH1 -160C/A variant in a promoter region for familial and sporadic forms of gastric cancer. The association of homozygote variant -160A/A with gastric cancer was shown for patients as with familial (OR=12,3; p=0,03) so sporadic form (OR=8,4; p=0,02). In addition it has been shown that the genotype 2076TT is associated with risk of a gastric cancer in a presence of a genotype -160CA for sporadic (OR=9,8; p=0,002) and the familial form (OR=12,3, p=0,009) but does not lead to the risk increasing in a presence of -160CC genotype. It is interesting that -160AC/2076TT genotype have not been found among 112 healthy controls.

In summary, mutations increasing cancer risk have been shown more frequent in MLH1 and MSH2 genes than CDH1. The genotype -160AC/2076TT of CDH1 has been certain for the first time as a risk genotype of gastric cancer.

#### P06.035

#### CDH1-160 polymorphism in Gastric cancer; Could be an informative marker?

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Introduction: Despite declining incidence rates, gastric cancer (GC) is a major cause of death worldwide and in Guilan Province, I.R. Iran. E-Cadherin is an adhesion molecule that is thought to be involved in gastric cancer patients. Polymorphism at position -160 C/A has been shown to affect GC risk in some studies.

Material and methods: DNA was extracted from peripheral blood cells according to the protocols. E-Cadherin polymorphism at position -160 C/A was investigated using PCR RFLP method.

Result: Fifty-two cases (17 gastric cancer and 35 of their healthy 1<sup>st</sup> relatives) were included in the study. Allele Frequency was 90.4 % for C and 9.6 % for A. 84.6 % of cases were Homozygote (CC in 43 cases and AA in 1 case) and 15.4 % were Heterozygotes (C/A in 8 cases). A>C polymorphism was seen in 17.3 % of cases (in homozygote or heterozygote state). This have seen in 23.5 % of those with gastric cancer and in 14.3 % of healthy one with gastric cancer in their 1<sup>st</sup> relatives

Conclusion: Polymorphisms in CDH1 have inconsistent relation with gastric cancer studies. In the present study, the CDH1-160C/A

(rs16260) polymorphism have higher frequency in affected cases versus their healthy related subject. Variation in allelic frequency in this study in contrast to other population studies, suggests that this SNP may be a good marker along with other SNPs.

Key words: Gastric cancer, polymorphism, CDH1-160, Iran

#### P06.036

##### Gastric and breast cancer occurrence in CDH1 mutated families

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Age-dependent penetrance of CDH1 mutations and relevance of clinical management guidelines are currently debated. Our objective was therefore to describe variabilities in CDH1-related carcinomas.

Sixteen families were identified with a deleterious CDH1 mutation between 1998 and 2008 in our laboratory. One mutation was found in 4 unrelated families, all others were unique. Fourteen index cases had a gastric cancer: 9 of diffuse type and 5 unspecified. Ages at diagnosis ranged from 21 to 63 years (mean = 38). Two index cases were free of gastric cancer but tested because of a bilateral lobular breast cancer at age 42 in one case, and a rectal linitis at 23 years of age in the other case. No family history of gastric cancer was found for 5 mutation carriers. Associated breast cancer was reported in 3 families (2 cases were specified as lobular breast cancer). In one family, 6 of 10 mutation carriers underwent prophylactic gastrectomy; all showed both invasive and *in situ* signet cell foci. However the oldest mutation carrier who declined gastrectomy was clinically asymptomatic at 64 years of age. In a second family, 13 members were tested for the familial mutation, and 2 of the 4 mutation carriers were free of cancer at ages 65 and 49 respectively.

The intrafamilial phenotype variability observed in our series indicates the need for international consortium to provide reliable data on penetrance of CDH1 mutations before validate guidelines for clinical management.

#### P06.037

##### IL-1 $\beta$ -511 T/C polymorphism does not contribute to GEP-NET susceptibility

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GEP-NETs represent a heterogeneous group of tumors, arising from diffuse endocrine system of gut and pancreas. Tumors are either solitary, or occur as a part of MEN-1 syndrome. The genetic basis of GEP-NETs is still largely unknown, but there is growing evidence that chronic inflammation through proinflammatory cytokines contributes to patients' susceptibility to acquire tumors. The role of IL-1 $\beta$  in the gastrointestinal tract inflammation and cancerosis has been extensively studied and *T* allele at -511-IL-1 $\beta$  promotor region was associated with aggravated inflammatory reaction measured through elevated IL-1 $\beta$  serum levels, higher gastric cancer susceptibility and worse prognosis. The aim of our study was to estimate allelic frequency for -511 promotor SNP in IL-1 $\beta$  gene in patients with GEP-NETs. DNAs obtained from 101 GEP-NET patients and 150 unrelated healthy volunteers were genotyped for the IL-1 $\beta$  -511 SNP using real-time PCR TaqMan<sup>®</sup> SNP genotyping assays. To compare the frequencies  $\chi^2$  test was used and results were significant if  $p < 0.05$ . Although high expression genotypes (T/C and T/T) and *T*-allele occurred more frequently among GEP-NET patients (63.37% vs. 56.67% and 38.61% vs. 35% respectively), there were no statistically significant differences in genotypes distribution ( $p = 0.5541$ ), high expression genotypes ( $p = 0.3530$ ) or in the allelic distribution ( $p = 0.4651$ ) between patients and controls. Although important in pathogenesis of gastrointestinal adenocarcinoma, IL-1 $\beta$  seems not to contribute to GEP-NET development.

#### P06.038

##### Molecular characterization of deletions of SDH genes in paraganglioma-pheochromocytoma patients identifies five novel deletions incl. the first Dutch SDHB founder deletion

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A major cause of paraganglioma/pheochromocytoma is germline mutation of SDHB, SDHC and SDHD.

MLPA analysis of 126 patients negative for point mutations in the SDH genes identified five deletions in SDHB, SDHC and SDHD, and additionally in TIMM8B and C11orf57.

An identical deletion of exon 3 of SDHB was identified in nine apparently unrelated patients.

One patient had a 10kb deletion of SDHD exons 1 and 2, of the entire TIMM8B gene and of exons of C11orf57. A second deletion of SDHD and TIMM8B gene was identified in one patient. Only molecular characterization differentiated the deletions in the above two patients.

Another patient showed a SDHD- MIRb -Tensin deletion-insertion. The final patient showed deletion of exons of the SDHC gene.

The identical deletion of SDHB and the common haplotype of carriers indicates that this mutation is the first Dutch SDHB founder mutation. The predominantly non-familial presentation of these patients strongly suggests reduced penetrance.

The deletions of TIMM8B and C11orf57 are the first to be described but do not appear to result in a phenotype.

#### P06.039

##### H-ras gene polymorphisms and sporadic colon cancer in Croatian population

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High incidence of colon cancer worldwide indicates the importance of exploring genetic alterations that lead to its carcinogenesis. Two polymorphisms in H-ras gene, hexanucleotide tandem repeats in the first intron and SNP T81C in the first exon, that might be connected with susceptibility to cancer have been described. The aim of our study was to investigate these loci in Croatian population and to determine if any of them is connected with susceptibility to colon cancer. Two hundred healthy volunteers and 200 colon cancer patients were genotyped using PCR and RFLP methods. Frequencies at hexanucleotide locus were 44.5%, 22.5%, 5.5%, 7%, 1% and 19.5% in healthy population, and 49%, 26%, 3.5%, 2.5%, 5.5% and 13.5% in colon cancer for P1/P1, P1/P2, P2/P2, P2/P3, P3/P3 and P1/P3 genotype respectively. Allele frequencies were 65.5%, 20.3% and 14.2% in healthy population and 68.8%, 17.8% and 13.5% in colon cancer for P1, P2 and P3 respectively. For SNP T81C locus frequencies were 42.5%, 42.5% and 15% in healthy population and 62.5%, 35% and 2.5% in colon cancer for TT, TC and CC respectively. Allele frequencies were 63.8 % and 36.2% in healthy population and 80% and 20% in colon cancer for T and C alleles respectively. Genotype P3/P3 was more common in colon cancer patients and P2/P2 and P2/P3 genotypes in healthy population. Allele -81C was more common in healthy volunteers in comparison to colon cancer patients.

#### P06.040

##### Contribution of the Rad50, Mre11 and NBN genes to hereditary breast and ovarian cancer

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The Rad50-Mre11-Nbs1 complex is essential for DNA repair. Rare genetic disorders are associated with homozygosity for mutations of the complex components; each including very high cancer risk. Heterozygotes may be at higher risk of breast cancer.

To evaluate the contribution of mutations and variants in these genes to familial breast cancer in France, we analyzed their full coding sequence in a series of non-BRCA HBOC cases. Cases were seen at the Oncogenetics consultation at the Centre Jean Perrin; written informed consent was obtained, and DNA purified from peripheral blood. 600 women with no personal or family history breast or ovarian cancer was

also studied.

In 317 cases, *Rad50* revealed two variants (Q426R, Q1011R) predicted to significantly disrupt protein structure or function. Q426R was observed in two families. These exons and exon 5, the site of a Finnish founder mutation, were analyzed in 600 controls: none of these variants were found, though one additional likely deleterious variant, N981I, was observed.

In 353 cases, *Mre11* revealed two deleterious mutations (R576X, R592X) and three likely benign variants (A492D, E497K, R576Q). One probably deleterious mutation was observed among controls: W210G concerns the same amino acid seen in ATLD patients homozygous for W210C. No variants of *NBN* were observed in 250 cases. Our results suggest that the R-M-N complex may contribute to breast cancer risk in a small proportion of non-BRCA HBOC families. The observation of deleterious mutations among the non-HBOC control group, however, suggests that the oncogenic penetrance of such mutations may be modest.

#### P06.041

#### Codon 72 Polymorphism of P53 gene is Associated with an Increased Susceptibility to Hepatocellular Carcinoma in the Turkish Population

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**Background and Aim:** The P53 tumor suppressor gene plays role major role in molecular mechanism of hepatocellular carcinoma (HCC) being involved in cell cycle control, the initiation of apoptosis and in DNA repair. The effect of p53 Arg72Pro polymorphism on HCC risk remains consistent due to ethnic differences of the populations studied. We aim to evaluate the p53 Arg72Pro polymorphism on the susceptibility of HCC in Turkish population. **Method:** In case-control study including 54 patients with HCC and 112 cancer-free control subjects matched for age, gender. P53 Arg72Pro polymorphism was genotyped using PCR-RFLP. Fisher's test with Woolf's approximation was used for statistical analysis. **Results:** The frequency of Pro allele was 49,1% in HCC cases and 33,9 in controls, respectively. The Pro allele was significantly associated with the presence of HCC (OR= 1,9, 95% [CI]= 1,17 - 2,99 p=0,01.). In addition, Pro/Pro homozygote genotype were more frequent in patients with HCC than controls (OR, 2,9; 95%CI, 1.242-6.851 p= 0,02). We found that allele and genotype frequencies of the control group for codon 72 of P53 are very similar to NCBI SNP database records for the European population (RefSNP ID:rs 1042522). **Conclusion:** These findings indicate that the Pro allele of p53 Arg72Pro polymorphism is associated with the presence of HCC and Pro/Pro homozygote genotype is a potentially one of the genetic risk factor for HCC in Turkish population. Carriage of Pro allele is a significant predictor for HCC and therefore it can be used as a biomarker for susceptibility to HCC.

#### P06.042

#### The Polymorphism of DNA repair gene XRCC3 Thr241Met Is Associated with an Increased Risk of Hepatocellular Carcinoma in the Turkish Population

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**Background and Aim:** Hepatocellular carcinoma (HCC) is associated with HBV infection and chemical carcinogens (such as aflatoxin B1) that induce DNA damage. In addition, genomic instability and DNA repair play important role in hepatocarcinogenesis. we tested the hypothesis that the polymorphism of DNA repair gene X-ray repair cross

complementing group 3 (XRCC3) Threonin (Thr)241Metionin (Met) is associated with risk of developing HCC.

**Method:** Genomic DNA was extracted from peripheral blood cells of 42 patients with HCC and 105 cancer-free control subjects matched for age, gender. XRCC3 Thr241Met genotypes were identified PCR-RFLP. Fisher's test with Woolf's approximation was used for statistical analysis.

**Results:** The frequencies of Thr allele was 83.7% in HCC cases and 55.7 in controls, respectively. The Thr allele was significantly associated with the presence of HCC (OR, 4,09, 95% CI, 2.131-7.876 p<0.0001). In addition patients with HCC had a significantly higher frequency of Thr/Thr homozygote genotype than controls (p= 0,01, OR, 2,7; 95%CI, 1.282-5.711). We found also a significant association between the Thr/Thr homozygote genotype and hepatitis B virus (HBV) positive cases ( p= 0.003, OR, 4.95; 95%CI, 1.740- 14.095).

**Conclusion:** These results provide evidence that the Thr allele of XRCC3 Thr241Met polymorphism is associated with the presence of HCC and Thr/Thr homozygote genotype is a potentially one of the genetic risk factor for HCC in Turkish population, especially HBV positive case. Carriage of Thr allele is significant predictor for HCC therefore it can be used biomarker for HCC

#### P06.043

#### Identification of a novel missense Mutation in the p53 gene in Iranian Patients with hepatocellular carcinoma

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The p53 gene plays a major role in hepatocellular carcinoma (HCC). Acquired mutations in this gene may provide clues to etiology. Some carcinogenic agents, such as aflatoxin are considered to be associated with specific genetic changes in the p53 gene. We attempted also to analyze the p53 mutations, especially in exons seven and eight, in tumor tissues from subjects suffering HCC in Iran.

A total of 25 archival formalin fixed paraffin embedded samples were collected that have been prepared between of 1997-2006 from hospitals in southwest and northwest Iran's. These samples have been diagnosed as HCC and classified into well differentiated (39%), moderately differentiated (54%), poorly differentiated (4.5%) and undifferentiated (2.3%), respectively. The hepatitis B virus (HBV) was detected in 16% (n=7) and 11% (n=5) of patient's sera that were affected with liver cirrhosis. No patient was infected with hepatitis C virus (HCV). We also examined the codon 249 within the exons 7 using RFLP as well the full length of exons 7 and 8 of p53 gene in the patients by SSCP. However, only in one patient was a mutation detected in codon 302 that has been afterward verified with direct sequencing. All other samples were negative for mutation in the above mentioned exons of p53 gene.

#### P06.044

#### Germline and Somatic CDH1 Deletions in Hereditary Diffuse Gastric Cancer

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Hereditary diffuse gastric cancer (HDGC) families carry *CDH1* heterozygous germline point or small frameshift *CDH1* mutations in 30% of the cases described to date. HDGC tumors acquire complete *CDH1* inactivation through *CDH1* promoter hypermethylation in 50% of the cases.

We hypothesized that *CDH1* genomic rearrangements would be found in the germline of *CDH1* negative HDGC families and somatically as the 'second-hit' in DGC of *CDH1* mutation carriers.

The germline of 93 *CDH1* mutation negative families was screened for large genomic rearrangements by MLPA and validated by RT-PCR. Breakpoints were cloned with oligo-CGH-arrays and long-range-PCR. *In-silico* analysis was used to determine a potential mechanism for rearrangements. Samples collected from 28 DGC (17 patients among 15 HDGC families) were analyzed for somatic *CDH1* mutations, LOH and promoter hypermethylation.

Seven percent of previously described mutation negative HDGC pro-

bands carried genomic deletions caused by mechanisms involving mainly non-allelic homologous recombination in Alu-sequences. Two families carried an identical deletion encompassing the full *CDH3* sequence and *CDH1* exons 1 and 2, most probably derived from a common ancestor. Other deletions affecting exons 1, 2, 15 and/or 16 were identified. *CDH1* somatic deletions (LOH) were found in 42.9% of 28 neoplastic lesions analyzed, adding LOH as a major 'second-hit' mechanism in HDGC tumors.

*CDH1* large deletions increase the susceptibility to HDGC when occurring in patients's germline; and determine DGC development when targeting the wild-type allele in *CDH1* germline mutation carriers' stomachs. These results are pivotal for HDGC management and treatment.

#### P06.045

##### Germline mutation of the E-Cadherin Gene in 3 sibling cases with advanced gastric cancer - Clinical consequences for the other family members

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**Background & Aims:** Germline mutations in the E-cadherin (*CDH1*) gene have been found in families with hereditary diffuse gastric cancer (HDGC). So far 68 distinct *CDH1* germ line mutations have been reported in HDGC. These families are characterized by a highly penetrant susceptibility to diffuse gastric cancer with an autosomal dominant pattern of inheritance. We describe the clinical presentation of 3 sibling cases with advanced gastric cancer, the way of confirming the suspicion of underlying HDGC and the clinical management of the other healthy family members according to the guidelines of the International Gastric Cancer Linkage Consortium (IGCLC).

**Methods:** Screening for *CDH1* germline mutation was done by denaturing high-performance liquid chromatography and automated DNA-sequencing. The clinical suspicion of HDGC has been confirmed by identifying a frameshift mutation in exon 9 (1302\_1303insA, 1306\_1307delTT) of the E-cadherin gene.

**Results:** Eight of 9 tested family members have been positive for the *CDH1* germline mutation. Prophylactic laparoscopic gastrectomies have been done in 5 mutation carriers. After pathological examination we could identify intramucosal malignant signet-ring cell carcinoma in all resected stomachs.

**Conclusions:** This report underlines, that prophylactic gastrectomy remains the only option to eliminate the high risk for gastric cancer in *CDH1* mutation carriers.

#### P06.046

##### Impact of high risk human papillomaviruses on the innate immune response of keratinocytes

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Infection of keratinocytes results in the production of antiviral cytokines via the activation of type I interferon (IFN) response genes and the attraction of the adaptive immune system via the production of pro-inflammatory cytokines, all activated after the intruding virus is recognized

by intracellular pathogen recognition receptors (e.g. Toll-like receptors, TLRs). We found that undifferentiated keratinocytes, which form the basal cell layer targeted by HPVs, express only one intracellular TLR, namely TLR3 (sensing viral RNA). A genome-wide gene expression study of poly I:C (TLR3 agonist) stimulated normal and hrHPV-infected keratinocytes showed a significant decrease in the activation of genes for type I IFN and pro-inflammatory cytokines (e.g. MCP1/CCL2, IL1B, IL6) in hrHPV-infected keratinocytes. In addition, genes encoding antigen presentation and anti-microbial molecules were downregulated in hrHPV-infected cells. The presence of hrHPV increased expression of cell cycle regulators, kallikreins, heat shock proteins, and tumor promoting cyto/chemokines. Results were confirmed by quantitative RT-PCR as well as by ELISA for cytokine production.

Our data suggest that hrHPV interferes with the regulation of innate and adaptive immunity induced by keratinocytes. These studies will yield candidate genes to study genetic polymorphisms that play a role in susceptibility to persistent infection with hrHPV.

#### P06.047

##### *IL10 (-1082G/A) polymorphism and cervical intraepithelial neoplasia*

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Genital Human Papillomavirus (HPV) is a common sexually transmitted infection. The genetic background and the host immune response are believed to be an important determinant in the relation between HPV persistent infection and carcinogenesis. The objective of this study was to examine the correlation between *IL10* (-1082) polymorphism and cervical intraepithelial neoplasia.

Hundred LSIL and HSIL patients and 50 normal controls were enrolled in this study. DNA was extracted from blood sample by the salting out method and PCR amplified. *IL10* and *IFN* genotyping was performed by the PCR-SSP (Polymerase Chain Reaction-Sequence Specific Primer) method, using the "Cytokine Genotyping Tray" (One-Lambda, Inc, Canoga Park, CA).

There was no statistically significant frequency difference observed between patients and controls ( $p=0.09$ ). Then the analysis was performed in the stratified sample, LSIL and HSIL versus controls. The GG genotype frequency in HSIL patients compared with control group frequency was statistically significant ( $p= 0.05$ ). The G/G genotype was significantly more common in control group, 18% versus 7.3%, OR= 2.79 (CI=95%). G/A and A/A genotype distributions were not different between LSIL, HSIL patients and controls.

This study suggests that IL-10 low levels may influence the cervical malignant progression. Further understanding of the role of cytokines may contribute, as a prognostic factor, for improved treatment for squamous intraepithelial lesions.

#### P06.048

##### Bilateral mucinous cystadenoma of ovary in a patient with 10q23 microdeletion including the *PTEN* and *BMPR1A* genes

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Juvenile polyposis syndrome (JPS) is a rare hereditary condition due to mutations in SMAD4 or BMPR1A genes. Multiple juvenile polyps can also be found in a related group of syndromes with multisystemic involvement including Cowden disease, Lhermitte-Duclos disease, Bannayan-Riley-Ruvalcaba syndrome and Proteus-like syndrome, all grouped as *PTEN* hamartoma tumor syndrome (PHTS). All forms of juvenile polyposis manifest in older childhood or early adulthood. Infantile juvenile polyposis is a rare entity, presenting in the first year of life with severe gastrointestinal symptoms. Many of these patients have associated macrocephaly, hypotonia and congenital anomalies. It was recently recognized that patients with infantile polyposis have 10q23 microdeletion, involving both BMPR1A and PTEN genes. There is a major risk for gastrointestinal malignancies in these patients, but risk for development of other tumors is not known. We describe the patient with history of infantile polyposis, macrocephaly, developmental delay, hypotonia and 10q23 microdeletion. At age 14 she presented with bilateral mucinous cystadenoma of the ovary. This type of tumor was not previously reported in association with JPS, PHTS or infantile

tile polyposis. We believe that ovarian cystadenomas is another neoplastic complication of infantile polyposis, and that our report widens a spectrum of 10q23 microdeletion phenotype.

#### P06.049

#### International consensus for neuroblastoma molecular diagnostics: Report from the international neuroblastoma risk grouping (INRG) Biology committee

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**Background:** Neuroblastoma serves as a paradigm for utilizing tumour genomic data for determining patient prognosis and treatment allocation. However, prior to the establishment of the International Neuroblastoma Risk Group (INRG) Task Force in 2004, international consensus on markers, methodology, and data interpretation did not exist, compromising the reliability of decisive genetic markers and inhibiting translational research efforts. The objectives of the INRG Biology Committee were to identify highly prognostic genetic aberrations to be included in the new INRG risk classification schema and to develop precise definitions, decisive biomarkers, and technique standardization.

**Methods:** The review of the INRG database by the INRG Task Force finally enabled the identification of the most significant neuroblastoma biomarkers. In addition, the Biology Committee compared different cooperative group standard operating procedures to arrive at international consensus for methodology, nomenclature and future directions.

**Results:** Consensus was reached to include MYCN status, 11q23 allelic status, and ploidy in the INRG Classification System based on an evidence-based review of the INRG database. Standardized operating procedures for analyzing these genetic factors were adopted and criteria for proper nomenclature were developed.

**Conclusions:** Neuroblastoma treatment planning is highly dependant on tumour cell genomic features, and it is likely that a comprehensive panel of DNA-based biomarkers will be used in future risk assignment algorithms applying genome-wide techniques. Consensus on methodology and interpretation is essential for uniform INRG classification and will greatly facilitate international and cooperative clinical and translational research studies.

#### P06.050

#### Study of primary and secondary tumors from patients with laryngeal and oropharyngeal cancer - a comparative approach

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Our study was focused on laryngeal and oropharyngeal cancers, which have nowadays an increased incidence, due to unhealthy habits like tobacco and alcohol consumption. We used transmission electron microscopy (TEM) in order to highlight the ultrastructural features of cancer cells, in primary and secondary tumors. The differences between the inner architecture of the tumor cells were correlated with the expression of some genes (oncogenes and tumor suppressor factors), in order to establish the aggressiveness of the tumor, in different disease stages.

Primary- and secondary tumor tissues, and also non-transformed tissue (from the vicinity of the tumor) were surgically obtained from 16 patients (8 with primary tumors and 8 with secondary tumors), from the "Ilfov" Emergency County Hospital, Bucharest.

The most important observation made from the ultrastructural data obtained by transmission electron microscopy is the aggressiveness and invasiveness of this type of cancer. The TEM conclusions are sus-

tained by the overexpression of the studied oncogenes, in both type of tumors, and also the overexpression of the studied tumoral suppressor genes, especially in primary tumors. *P16* gene was underexpressed in the tumor cells, compared to normal ones, in only one case (carcinosarcoma tumor type). *P21* gene level is decreasing in the secondary tumors, compared to primary ones.

The studied genes proved to be good candidates as tumoral markers in laryngeal and oropharyngeal cancer. The analysis of the combinations between the expression level of these genes is of real relevance for the prognosis of patient evolution and improvement of the treatment strategies.

#### P06.051

#### LATS2 tumour specific mutations and methylation of promoter in non-small cell carcinoma

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LATS2 is putative tumour suppressor gene, involved in maintenance of cell stability. It is situated on a chromosome location, which is in tumours often affected by LOH. Diminished expression is detected in different types of cancer and often related to methylation.

Fifty-one adenocarcinomas (ADC) and sixty-seven squamous cell carcinomas (SCC) in different tumour stages and adjacent healthy lung tissue were included in our study. LATS2 alterations were discovered with denaturising high-pressure liquid chromatography (DHPLC). Gene expression levels were established with real-time PCR and methylation with restriction analysis of bisulphite treated DNA. Results from DHPLC and methylation analysis were confirmed by sequencing. LATS2 was down regulated in ADC and SCC (Student's t test,  $p < 0.05$ ). Nine different genetic alterations were observed in the LATS2 gene; including four novel intron polymorphisms, one novel missense mutation (S1073R) and one novel PAPAP repeat deletion (del 472\_479 APA-PAPAP).

We were first to describe mutations in LATS2, significantly related to SCC in an advanced stage of development. Analysis of promoter methylation revealed that all of the tumours with diminished LATS2 expression had recognition sites methylated.

Expression of LATS2 gene in ADC and SCC was diminished and related to promoter methylation. We can conclude that promoter LATS2 methylation is frequent and early event in lung tumourigenesis, found already in stage I tumours (SCC and ADC). Mutations are obviously more specific and late events in carcinogenesis, found mostly in advanced SCC tumours.

#### P06.052

#### First LFS family with childhood rib chondro-sarcoma and novel p53 germ-line mutation

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A novel p53 germ-line mutation in an Austrian family with Li Fraumeni Syndrome (LFS) is reported here. The mutation was identified as a missense mutation in exon 10 at c.1025G>C by direct sequencing of the entire coding sequence of p53. This constitutional mutation leads to an amino acid substitution in the protein changing arginine for proline (p.R342P) and occurs in a region that is responsible for oligomerization. A review of mutational p53 database UMD-p53 (Beroud and Soussi, 2007) revealed only 4 cases of sporadic malignancies, namely an advanced breast carcinoma, one ewing's sarcoma and two ovarian cancers that harbored this genetic alteration demonstrating this variant to be pathogenic and extremely rare even in somatic malignancies. Affected members of the here reported LFS family (LF-342-1) developed the classical spectrum of malignancies and carried all the identical gene alteration, indicating its transmission by inheritance and suggesting this mutation to severely impair the physiologic function of p53.

**P06.053****Search for alternative genetic defects in families suggestive of the Li-Fraumeni syndrome with no identified missense mutations in the TP53 gene**

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Li-Fraumeni syndrome (LFS) is a disorder characterised by autosomal dominant predisposition to a broad spectrum of cancers. Most LFS patients carry missense germline mutations in the tumour suppressor gene TP53. Such mutations can be found in about 70 % of families conforming to stringent criteria of LFS, and in about 20 % of families conforming to less strict Li-Fraumeni - like (LFL) or Chompret criteria. Most mutations are located in the middle part of the TP53 gene encoding the DNA-binding domain (exons 5 - 9), and often only these exons are analysed in routine TP53 testing.

In search for the genetic basis of cancer predisposition in families suggestive of LFS but with no identified TP53 mutations in exons 5 - 9 we focused primarily on other defects possibly affecting the TP53 gene function. First, we completed the analysis of all TP53 exons by sequencing also the outer exons (26 families). Second, we used the MLPA method to exclude single- or multiexon deletions in the TP53 gene (7 families). Third, we applied bisulphite sequencing to analyse possible epigenetic silencing of one of the alleles of the TP53 gene by promoter methylation (14 families). Finally, we searched members of several families for mutations in the CHEK2 and SNF5 genes, which have also been associated with LFS in several rare families published in literature. However, none of these efforts revealed any molecular defects which could be responsible for hereditary cancer predisposition in the families analysed. Supported by grants MSM0021620813 and MZO00064203.

**P06.054****Association between manganese superoxide dismutase gene polymorphism (Ala-9Val) and cutaneous squamous cell carcinoma risk**

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Superoxide dismutase play an important role in the detoxification of superoxide radicals thereby protecting cells from damage induced by free radicals. One of the several metabolic pathways involved in carcinogenesis is the polymorphism in the mitochondrial targeting sequence (MTS) Val-9Ala of the MnSOD gene which influences the transfer and accumulation of the MnSOD enzyme into the mitochondria. Thus, the goal of this study was to find a relationship between MnSOD Val-9Ala polymorphism and squamous cell carcinoma (SCC) risk. A study of 91 patients with squamous cell carcinoma and 58 normal subjects was performed. The MnSOD MTS genetic polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism technique using NgoMIV and automated sequencing. No significant difference was found in cutaneous squamous cell carcinoma susceptibility in the subjects with Ala/Ala and Val/Ala genotype compared with Val/Val genotype (Odds ratio, 1.49; 95% confidence interval, 0.73-2.76; p= 0.3735). These data suggest no correlation between MnSOD Ala-Ala and MnSOD Val-9Ala genotypes and squamous cell carcinoma (SCC) risk.

**P06.055****Correlation of MDR1 C3435T polymorphism and expression in Iranian breast cancer patients**

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Multidrug resistant (MDR) is one of the problems in treatment of cancer patients by chemotherapy. One of the mechanisms responsible for drug resistance is over-expression of ABC-transporter genes such as MDR1. MDR1 encodes p-glycoprotein (p-gp), a transmembrane glycoprotein that transports many hydrophobic substrates and anti-cancer drugs from cell. Polymorphism in MDR1 gene may affect the expression level of the Pgp and subsequently can result to drug resistance. In this study we investigated the possible association between MDR1 gene C3435T polymorphism and MDR1 expression in Iranian breast cancer patients.

DNA and RNA were extracted from tumor and blood cells. For cDNA synthesis, 1 mg of total RNA from each sample was used to synthesize first-strand cDNA. Evaluation of the expression level of MDR1 was performed by Real-Time Quantitative PCR and PCR-RFLP was used for the detection of C3435T single nucleotide polymorphism.

A statistically significance in MDR1 expression was observed when samples from breast cancer patients were compared with healthy individual. We observed no difference in frequency of C3435T polymorphism between breast cancer patients and healthy controls. Clinico-pathological parameters of patients with breast cancer were compared for C3435T polymorphism. These data suggest that a number of other factors in addition to gene polymorphisms, e.g., gene amplification, promoter demethylation might be responsible for MDR1 over-expression in these patients.

**P06.056****Establishment of Study Group of Multiple Endocrine Neoplasia in Japan (MEN Consortium of Japan)**

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Multiple endocrine neoplasia (MEN) is a rare hereditary syndrome characterized by neoplastic disorder of endocrine organs. MEN is subdivided into MEN1 and MEN2, and the latter includes MEN2A, MEN2B and familial medullary thyroid carcinoma. MEN1 and MEN2 are caused by germline mutations of the responsible genes, MEN1 and RET, respectively, and mutations can be identified in most patients. Clinical guideline for diagnosis and management of MEN has been published in 2001, and that is widely accepted as a "golden standard". However, its contents are not based on reliable evidences. Efforts to establish clinical evidence are currently ongoing in Europe, USA and other regions of the world. In Asian countries including Japan, such efforts have not been initiated. Also, some have claimed that clinical courses of Japanese (Asian) patients may not be the same as that of Caucasian patients, which is not verified. To construct a reliable database of Japanese patients with MEN, we established a study group of MEN and named it "MEN Consortium of Japan". Each participant deposits anonymized clinical data of patients and those are cumulated in data handling center. Participants can freely extract clinical data from database for analysis from various respects. This database also enables long-term follow-up of patients even if they move from one hospital to another. As of February 2009, 21 major institutions have joined this Consortium and additional 20-30 institutions are expected to join in near future. This consortium will help clarify current status of diagnosis and clinical management of MEN in Japan.

**P06.057****Methyl-binding domain (MBD) protein based isolation of methylated DNA from human serum**

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DNA methylation testing of serum-DNA for minimal invasive testing of cancerous disease is of great diagnostic interest. Isolation of cell-free DNA from human serum is a big challenge because only nanogram amounts of DNA are present in one ml of serum.

In this work we tested the MBD-protein to isolate and enrich methylated DNA already during the DNA isolation step. Selective binding of cell-free serum DNA onto immobilised MBD directly in the serum would enable favorable concentrations of DNA for further analysis.

In addition DNA binding under native conditions preserves proteins for additional proteomic or immunological analyses.

Upon recombinant expression the MBD protein was attached to nickel beads obtaining binding of 1.35 mg MBD protein per ml of NiNTA-bead suspension (50%). After optimization DNA-binding capacity of immobilised MBD was tested in buffer and human serum; using 1 $\mu$ g of spiked-in human DNA we could retrieve 30% of the DNA from serum and about 75% from buffer. Binding specificity towards methylated DNA was evaluated by PCR upon methylsensitive restriction enzyme digestion, using several known differentially methylated genomic DNA regions. Although published, MBD did not selectively bind methylated DNA in a range of 0.1-0.8 M NaCl, which might be due to immobilization. Performing the DNA isolation from serum using a commercial kit, 10-17ng DNA per ml serum could be isolated and using the MBD-based strategy about 5ng/ml were obtained. Thus here we show that in principle DNA isolation from serum can be achieved using MBD-protein but binding is not selective for methylated DNA.

#### P06.058

#### Gene network and canonical pathway analysis in hematopoietic and soft tissue originated malignancies: A microarray experience of Medical Genetics Department of Kocaeli University in 2007-2008.

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Background: We performed gene expression analysis in hematopoietic tissue, ovarian cancer, prostate cancer, cervical cancer; breast cancer, endothelial cell lines, preeclampsia and HELLP syndrome, using microarray technology in University of Kocaeli.

Materials and Methods: ABI (Applied Biosystems, Foster City, CA, US) and Agilent (Agilent Technologies, Palo Alto, CA) platforms were used as microarray chips. Obtained data were analysed by using GeneSpring (GeneSpring 6.1, Silicon Genetics, Redwood City, CA) and Ingenuity Pathway Analysis (IPA) (Ingenuity Systems, Mountain View, CA, USA) software programmes for gene network and canonical pathway analysis. Array results were confirmed using Quantitative Real Time PCR (LightCycler, Roche Diagnostics GmbH, Mannheim, Germany) and TaqMan® Low Density Array Human Apoptosis Panel (TaqMan®, Applera, Norwalk, U.S.A.).

Results: Our results represents the first gene network analysis in Turkey. Here we define the importance of bringing samples to the microarray laboratory in safe conditions and value of RNA integrity number.

Conclusion: This technology is very useful to suggest new pathognomonic- prognostic markers and new therapeutic targets.

#### P06.059

#### Expression and functional analyses of miR-125b in breast cells

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Genomic instability is a common event in breast cancers. Various chromosomal and segmental loss or amplification regions have been detected in primary breast tumors and breast cancer cell lines. MicroRNAs are ~18-24 nt long non-coding RNAs that regulate protein expression by binding to target mRNA sequences in the 3' untranslated regions. A large number of microRNAs are localized to genomic instability regions in cancer cells. We screened for genomic gain/loss of 36 microRNA genes localized to common genomic instability regions in breast cancer cells. Among these, miR-125b-1 is localized to a loss region, 11q24.1, with variable amplification/loss levels. We detected fold number decreases compared to an internal PCR control in SUM-229, MDA-MB-435 and MDA-MB-361, whereas amplification was seen in some other cells (T47D, MDA-MB-468). RT-PCR analysis suggested lack of miR-125b-1 precursor transcript in MCF-7 cells, with no genomic fold number changes. To understand the possible role of this miRNA in breast cancer cells, we cloned the hairpin structure into pSUPER vector to stably transfect MCF-7 cells. Cells surviving anti-

otic selection were expanded and stable transfection was confirmed. Initial studies suggested a decreased proliferation rate in miR-125b transfected MCF-7 cells compared to controls. Further functional studies are underway to better understand the consequences of loss of miR-125b in breast cancer cells.

#### P06.060

#### MiRNA profiles in neuroblastoma: a link to functional studies?

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Background: neuroblastoma (NB) is an embryonal tumor originating from neural crest-derived undifferentiated cells that is characterized by variable clinical courses ranging from spontaneous regression to therapy-resistant progression. Recent studies suggested differential expression of miRNAs in neuroblastoma subtypes. However, the full repertoire of miRNAs expressed in NBs is not yet available. Furthermore their functional role in NB tumorigenesis remains elusive.

Methods: small RNA cloning, overexpression of miRNAs, clonogenicity assay, western blotting.

Results: miRNA libraries provide informative profiles of miRNA expression; the most abundant species were miR-125b, -21, -124a, -16 and 17-5p. Several new miRNAs are found within 3p25, 9p21, 11q14, 3p12, which represent sites of frequent genomic alterations in neuroblastoma. mir-885 on 3p25 and mir-331 on 12q22 were chosen for functional characterization. A mir-885 mimic inhibits growth of several NB cell lines triggering cellular senescence. In contrast, mir-331 slightly upregulates proliferation. Stable expression of mir-885 but not mir-331 inhibits growth of SH-EP and KELLY cell lines.

Conclusions: we provide evidence for several new human miRNAs; some novel miRNAs are within neuroblastoma-relevant chromosomal regions. mir-885 on 3p25 is a new tumor suppressor candidate.

#### P06.061

#### The RET mutations in Russian families with MEN 2 cancer syndromes: molecular diagnostics and prophylactic treatment

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Activating germline mutations of the RET protooncogene (10q11.2) have been identified as the underlying cause of uncommon heritable cancer syndromes of multiple endocrine neoplasia (MEN) type 2A, familial medullary thyroid carcinoma (FMTC), and MEN type 2B. Peripheral blood DNA was analyzed from 32 individuals of 13 families with MEN 2 applied for the help to the Russian NN Blokhin Cancer Research Center from January 1997 to December 2008. Genetic screening was performed by using PCR, restriction enzyme analysis, SSCP analysis and automatic sequencing for exons 10, 11, 13, 14, 15 and 16. Missense mutations of the RET gene were revealed and identified in all probands studied and in 11 asymptomatic relatives. Five types of mutations were found in 10 MEN 2A families: C634R (54.6%), C634G (18.2%), C634F (13.6%), C634Y (9.1%), C634W (4.6%). In two families with FMTC we found mutations C620R and S891A. RET mutation M918T was found in two patients from one family with MEN 2B. Moreover 61 Russian patients with sporadic medullary thyroid carcinoma (MTC) were tested for the most common MEN 2B and MEN 2A associated germline RET mutations. As a result, de novo RET mutations were revealed in 11 individuals with "sporadic" MTC.

Preventive thyroidectomy was performed in 6 asymptomatic carriers of RET mutations from 5 families with genetically and clinically confirmed MEN 2A syndrome. Thus molecular analysis enables to identify asymptomatic individuals at risk for MTC in early stage and to prevent or cure from cancer.

#### P06.062

#### Evaluation of mutS based mismatch detection for paralleled mutation testing on standard DNA-microarrays

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Mutation screenings for confirmation of suspected diagnoses and for verification of carrier status are an essential part of human genetic diagnostics. The to-date "gold standard" in mutation analysis is direct DNA sequencing, that allows analysis of single gene areas up to lengths of several hundred bases. Analysis of many gene areas or several complete genes for diagnostic reasons is very labor-intensive. Thus tests enabling paralleled analysis at low cost and high flexibility would be favourable.

The aim of this work was to evaluate the suitability of the mutation-specific mutS protein for a DNA-microarray based mutation detection method which enables highly-parallelized mutation detection. *E. coli* mutS protein recognizes single point mutations, as well as insertions and deletions of up to four base pairs and is thus usable for point mutation testing. Using different recombinant mutS-constructs however failed reliable mutation-testing on a DNA-microarray. However here-with we provide a convenient control system for testing "Enzymatic Mutation Detection" which could be helpful when trying other protein-based mutation tests upon DNA-chip hybridization using CEL1 endonuclease, EndoV, bacteriophage resolvase T4 endonuclease VII and T7 endonuclease I.

Keywords: mutation testing, mutS, microarray

#### P06.063

#### Increased risk of MB in heterozygous carriers of NBN gene germline mutations

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Cerebellar medulloblastoma (MB) is the most common highly malignant, invasive embryonal brain tumor in children. Several signaling pathways are known to be engaged in hereditary and sporadic MB. Nibrin, a protein product of the NBN (formerly NBS1) gene is a component of the Mre11/Rad50/Nbs1 complex that is critical for sensing and processing double strand breaks in DNA, and is also required for the proper initiation of base excision repair. Hypomorphic mutations in the NBN gene are the cause of Nijmegen breakage syndrome (NBS), a severe disease predisposing to different types of cancer, including MB. The aim of our study was to identify NBN gene mutations and to determine their frequency in Polish patients with different types of sporadic medulloblastomas. A group of 110 patients with MB was screened for mutations in the NBN gene by SSCP-PCR followed by direct DNA sequencing. Seven heterozygous carriers of two various germline NBN gene mutations (6.36%) were found: four with mutation c.511A>G in exon 5 and three with mutation c.657\_661del5 in exon 6. The risk of medulloblastoma was estimated as 2.89 (for c.511A>G) and 5.08 (for c.657\_661del5) times higher than in the general Polish population ( $p<0.05$ ). Our report is the first to document the frequency of heterozygous NBN germline mutations in pediatric patients with different types of medulloblastoma. Further investigations concerning a larger group of patients are necessary to assess the role of germline NBN mutations in predisposition to childhood medulloblastoma.

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#### P06.064

#### NBS1 gene mutations as a cancer risk factor.

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MRE11, RAD50 and NBS1 (MRN) complex is involved in DNA repair and cell cycle checking signaling. Molecular variants of *NBS1* gene may therefore constitute cancer risk factor. Heterozygous carriers of the *NBS1* 657del5 mutation have been shown to have an increased risk for melanoma, breast, colon and rectum cancer. Other studies have found no association between *NBS1* gene mutations and lymphomas. The aim of the study was to analyze the frequency of *NBS1* gene mutations by screening all 16 exons of this gene along with polymorphisms examination. DNA was isolated from PBL of 135 children with ALL, 270 women with breast cancer, 176 patients with larynx cancer, 93 with second primary tumors of head and neck, 131 with colorectal car-

cinoma and 1274 healthy individuals. I171V mutation of *NBS1* gene was the most frequent and has been found in 23 patients compared to only 8 in healthy individuals. Other mutations of the *NBS1* gene have been observed in lower frequencies. Genotyping data from the six polymorphic loci in *NBS1* gene, were used to impute haplotypes. Two of the evaluated haplotypes were associated with significantly increased leukemia risk ( $P=0.0038$ ,  $P<0.0001$ ). Since DNA was isolated from non-malignant cells, all mutations found in cancer patients appeared to be of germline origin. It can be concluded that I171V mutation of *NBS1* gene is associated with predisposition to malignancies and *NBS1* allele I171V may be a general cancer susceptibility gene of low or middle risk.

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#### P06.065

#### Differential effects of Nucleostemin knocking-down on cell cycle arrest and apoptosis in the bladder cancer cell lines 5637 and SW1710

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The recently identified *Nucleostemin* (NS) gene encodes a nucleolar protein expressed mainly in adult and embryonic stem cells, which may interact with p53. Nucleostemin is also thought to regulate the proliferation of cancer cells, but the mechanisms involved are poorly understood. Therefore, we have investigated NS expression in a series of bladder carcinoma cell lines and in normal uroepithelial cells. High NS expression was found in several bladder carcinoma cell lines, but surprisingly moderate expression in normal uroepithelial cell cultures as well. Knock-down of NS expression by siRNA induced apoptosis significantly and caused a severe decline in cell proliferation in both the 5637 and SW1710 cell lines. However, apoptosis was more strongly enhanced in 5636 cells, with mutant *TP53* and *RB1* but wild type *CDKN2A/p16<sup>INK4a</sup>*, than in SW1710 cells, with mutant *TP53* and *CDKN2A* but wild type *RB1*. Moreover, NS-siRNA treated 5637 cells accumulated mainly in the G2/M phase, whereas SW1710 cells arrested in G0/G1. Our data indicate that NS expression is necessary for cell proliferation and evasion of apoptosis in bladder cancer cells, independent of its effect on p53. In other cell lines, suppression of NS has been reported to cause alternatively G2/M or G0/G1 arrest. Our data suggest that the precise effect of NS on cell cycle regulation may depend on the functional status of *RB1* and *CDKN2A/p16<sup>INK4a</sup>*.

#### P06.066

#### Functional gene polymorphisms that are strongly associated with risk for oral cancer

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Recent evidence indicated that gene polymorphisms are associated with increased risk for oral squamous cell carcinoma (OSCC). We investigated the combinatory effect of 31 inherited functional DNA polymorphisms in genes of factors related to angiogenesis, inflammation and thrombosis in an attempt to predict the occurrence of OSCC in Europeans.

DNA samples were isolated from blood of 162 OSCC patients and 168 healthy controls of comparable age, gender, and ethnicity (Greeks and Germans). Polymorphisms were investigated in genes that encode: cytokines and their receptors (IL-1b, -4, -6, -8, -10, -18, TNF- $\alpha$ , - $\beta$ , VEGF, Leptin, Leptin receptor), matrix metalloproteinases and their inhibitors (MMP-1, -3, -7, -9, -13, TIMP-2), platelet glycoproteins and coagulation factors (GPIa, GPIba, PAI-1, AGT, ACE, TAFI, Thrombomodulin, Protein Z, SDF1, Factors II, V, XII, XIII and MTHFR). A series of multivariate regression models (adjusted for age and gender) was constructed in order to assess the contribution of homozygous or heterozygous variant polymorphic genotypes upon overall, early and advanced stages of OSCC development.

The contribution of TNF- $\alpha$  and IL-6 polymorphisms was consistent and robust in all regression models. When the mode of inheritance of each

variant allele was taken into account, five polymorphisms emerged as primary predictors for all OSCC stages: TIMP-2 (OR=26.33), TNF- $\alpha$  (OR=15.27), IL-6 (OR=8.33), IL-8 (OR=3.54) and IL-10 (OR=2.65). The contribution of these five factors in the occurrence of OSCC is highly significant. Three of them increase risk more than tobacco (8-26 times versus 6 times, respectively). Based on these findings, possible interactive mechanisms of implicated factors leading to OSCC development and an algorithm of risk estimation will be presented.

#### P06.067

#### COX-2 -765 G > C functional promoter polymorphism and its association with Oral squamous cell Carcinoma.

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Cyclooxygenase-2 (Cox-2) is a key enzyme in the conversion of arachidonic acid to prostaglandins that has been shown to have a particular importance in the progression of several malignancies including OSCC. In the current report, we designed a case-controlled study to evaluate the susceptibility and prognostic implications of the functional -765 G > C genetic variation in OSCC patients. A PCR and restriction fragment length polymorphism analysis was used to determine the polymorphism in Indian population of patients with OSCC ( $n = 120$ ) and in healthy control subjects ( $n = 100$ ). Genotype frequencies of Cox-2 G765G, G765C and C765C were 77.5%, 15.83% and 6.66% in the cancer patients and 91%, 7% and 2% in the controls, respectively. Cox-2 G765C genotype is significantly associated ( $p$ -value = 0.04) with OSCC patients when compared with controls. G765C genotype was a 1.48-fold increased risk for OSCC. G765C genotype is statistically significant with Chewing habitual risk factor ( $p=0.002$ ). This is the first report from India on the studies of COX-2 SNPs in Oral squamous cell carcinomas and our data suggest that this genetic variant may play a role in mediating susceptibility to OSCC cancer.

#### P06.068

#### Romanian ovarian cancer research for establishing a model of tumors progression and metastasis evolution

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In Romania, ovarian cancer is the second most frequent cause of death among women consecutive to a malignancy. Due to the lack of clinical symptoms, ovarian cancer is often discovered in late stages and it is the gynaecological malignancy with the worst prognosis. Another explanation for this poor prognosis is the absence of screening tests which would allow an early diagnosis. Early diagnosis makes the difference up to a 95 percent survival rate if the ovarian cancers are diagnosed in an early stage and only 20 percent survival if the patients are in stage III or IV. The genetic changes and molecular mechanism underlying these tumors remain poorly understood. Department of Medical Genetics of University of Medicine in Timisoara had initiated a research program for ovarian cancer. In this study are included patients diagnosed with ovarian tumors in the Western part of Romania. The study implies DNA ploidy analysis using fluorescence in situ hybridization (FISH) correlated with DNA copy number changes detected by comparative genomic hybridization (CGH) and clinical outcome. The aim is to develop a model for the progression of ovarian cancer by assessing the tumor development and progression from the benign through borderline to malignant ovarian tumors and identification of a genetic profile associated with the cancer aggressiveness and metastasis.

#### P06.069

#### A significant loss of leucine rich repeat domain in a novel candidate tumour suppressor gene PHLPP in cancer: Expression studies

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PTEN and a novel tumour suppressor, PHLPP are the negative regulators of Akt signalling. In this study, the expression of four major func-

tional domains, PH domain, leucine-rich repeat region (LRR), PP2C-like catalytic core and PDZ binding motif of PHLPP gene together with PI3K/Akt pathway genes;Akt-1, PTEN and caspase-3 were examined in pediatric Acute Myeloid Leukemia (pAML) patients. The expression studies were performed by qRT-PCR in 35 pAML patients and in controls, CD33+ blasts isolated from healthy bone marrows. The results revealed that Akt-1 was up-regulated in pAML patients ( $p=0.06$ ). PTEN, PHLPP and caspase-3 were found to be decreased in pAML patients compared to controls (3 times ( $p>0.05$ ), 10 times ( $p>0.05$ ) and 3 times ( $p>0.05$ ) respectively). Expression of PH, PP2C-like catalytic core and PDZ binding domains were detected in our study group. Interestingly, expression of LRR in pAML patients was not detected. Amplification of PHLPP mRNA covering the region between exon 2 to exon 17 in pAML samples lacking LRR expression resulted in three different transcript variants. Direct sequencing results revealed a single nucleotide change in exon 5 at position 55. PHLPP functional domain expressions were also studied in various tumour tissues (colon, stomach, pancreas and breast tumours). In tumour samples, LRR and PP2C-like catalytic core expression were lost. PHLPP mRNA transcript variants different than those observed in pAML samples were detected in tumour samples. Western blot analysis results also confirmed the loss of non-truncated PHLPP protein in pAML patients and tumour samples. It can be proposed that PHLPP gene might act as a tumour suppressor in AML leukogenesis and tumorigenesis.

#### P06.070

#### Impact of genetic polymorphisms on a training-induced adaptation of the heart (The athlete's heart)

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**Background:** Athlete's heart, an adaptation to long-time and intensive endurance training, shows considerable individual differences. Genetic polymorphisms in cardiologic relevant signalling pathways seem to have an essential influence on the extent of physiological hypertrophy.

**Objective:** Analysis of polymorphisms in genes of the insulin-like growth factor 1 (IGF1) signalling pathway and whose negative regulator myostatin (MSTN), and the renin-angiotensin-aldosterone system (RAAS), and their relation to left ventricular mass (LVM) of endurance athletes. **Methods:** In 110 elite endurance athletes or athletes with a high amount of endurance training (75 males and 35 females) and 27 controls, which were examined by echocardiographic imaging methods and ergometric exercise-testing, the genotypes of 16 polymorphisms in 14 analysed genes were determined. Additionally, a mutation screen of the MSTN gene was performed.

**Results:** The polymorphisms in the IGF1 and the IGF1R gene showed a significant correlation to the LVM (IGF1:  $p=0.003$ ; IGF1R:  $p=0.01$ ). The same applies to a so far unnoticed polymorphism in the MSTN gene, whose mutation allele appears to increase the myostatic effect ( $p=0.015$ ). Contrary to a pathological hypertrophy, polymorphisms in genes of the RAAS seem to have generally no significant influence on a physiological hypertrophy. An important exception was a polymorphism in the aldosterone synthase gene (CYP11B2 C-344T;  $p=0.02$ ). Moreover, combinations of some polymorphisms showed significant synergistic effects on the LVM. Unexpectedly, these effects were only detected in male athletes.

**Conclusions:** We argue for the importance of selected polymorphisms in these cardiologic relevant signalling pathways on the degree of physiological hypertrophy of male athletes.

#### P06.071

#### Primitive neuroectodermal tumor (PNET) located in third and fourth ventricles and frontal lobe: case report of a 51-years-old woman

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Primitive neuroectodermal tumor (PNET) is rare but a highly malignant tumor of the central nervous system. PNET is usually described as a tumour of children being three fourths of these tumors appear in children younger than 15 years, and 50% are seen in the first decade of life. A second, smaller peak occurs in young adults (aged 21-40 years). PNET is classified into two types, based on location in the body: peripheral PNET and CNS PNET. It is a term for a group of small, round cell tumors of the central and peripheral nervous system thought to be derived from fetal neuroectodermal precursor cells.

We report on a 51-years-old woman with primitive neuroectodermal tumor located in third and fourth ventricles and right frontal lobe. The diagnosis was made in accordance with clinic, radiological and laboratory investigations.

Immunohistochemically, tumor cells were immunoreactive for synaptophysin, chromogranin A, some tumor cells immunoreactive for CD99. There were no immunoreactive cells for GFAP and vimentin CK7.

CDKN2A homozygous deletion study: paired blood DNA and PNET tumor tissue were investigated for CDKN2A deletion. We separately amplified 1 $\alpha$  and 2 exons of p16(INK4a) tumor suppressor and exon 1 $\beta$  of p14(ARF) for homozygous deletions. There were no homozygous deletions observed neither for p16 (INK4a) nor for p14(ARF) tumor suppressors studied.

All case review will be made in poster.

#### P06.072

#### CYP1B1 polymorphic variants associated with prostate cancer risk in Bulgaria

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**Background:** The CYP1B1 gene product is a member of the cytochrome P450 enzymes involved in the androgen metabolism and one of its tasks is catalysis of testosterone hydroxylation. Several studies indicate that common polymorphic variants may increase the activity of the enzyme and have a role in human prostate carcinogenesis.

**Materials and methods:** We have investigated the association with prostate cancer (PC) risk of four polymorphisms in exons 2 and 3 of CYP1B1 in a case-control study of 114 PC patients and 97 control individuals with benign prostate hyperplasia and normal PSA level. The polymorphisms were genotyped by direct sequencing.

**Results:** The strongest association with PC risk was demonstrated by D449D, where the presence of either one (OR=1.4, 95% CI = 0.9-2.1; p=0.052) or two C alleles (OR=1.8; 95% CI = 1.0-3.2; p=0.020) leads to increased risk. The CC genotype occurred in higher frequency in patients (50%) than in the controls (35%). Similarly in terms of L432V, the frequent CC genotype (50% and 37% among patients and controls, respectively), was associated with more than 1.5 fold PC risk (OR=1.69, 95% CI = 1.0-2.9; p=0.041). The polymorphisms N453S and A119S did not show any significant association with PC risk.

**Conclusions:** Regarding the androgen metabolism at least several studies have demonstrated the association between one or more genetic variants of CYP1B1 gene and increased PC risk. It appears that CYP1B1 polymorphisms D449D and L432V are associated with increased PC risk in the Bulgarian population.

#### P06.073

#### Enhancement of the efficacy of Docetaxel with Conjugated Linoleic Acid in LNCaP prostate cancer cells

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Prostate cancer is among the most commonly diagnosed cancers. The use of chemotherapy in the treatment of prostate cancer has shown promising results in improving the overall survival rate. The taxane docetaxel (Taxotere) has proved efficaciously in controlling tumour

progression by interfering with microtubule dynamics. Currently drug supplementation in cancer therapy has resulted in improved response, by minimising its toxicity. The  $\omega$ -6 fatty acid conjugated linoleic acid (CLA) has raised interest as an effective tumourcidal agent without inducing systemic harmful effects. The present study investigates whether CLA supplementation would enhance the efficacy of docetaxel in androgen sensitive LNCaP prostate cancer cells *in vitro* and seeks possible genetic alterations in this process. LNCaP cells were exposed to CLA followed by concurrent treatment with docetaxel for 24 and 48 hours. The cell viability was determined by MTT assay. Results showed that higher concentrations of CLA enhance the efficacy of docetaxel at 48 hour treatment time. Based on the involvement of NF- $\kappa$ B mediated apoptosis, four genes of this pathway were selected. From RT-PCR analysis, it was observed that CLA supplementation reduced the expression of MAP2K4, MAX, AKT1 and FADD compared to the effect of docetaxel on these genes. It is proposed that CLA supplementation reduces the proliferatory activities of LNCaP cells possibly by docetaxel-induced stress, resulting in the net antiproliferatory activities of docetaxel not being opposed. The notion that supplementation of chemotherapeutic drugs by fatty acids render cancerous cells to undergo cell death more efficaciously, potentially sustains beneficial prospects in cancer therapy.

#### P06.074

#### Study of gene expression in prostate cancer samples

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We determined the changes in gene expression in PCA tissues and to compare them to those in non-cancerous samples. Prostate tissue samples were collected by needle biopsy from 21 PCA and 10 benign prostate hyperplastic (BPH) patients. Total RNA was isolated, cDNA was synthesized, and gene expression levels were determined by microarray method (ABI, USA). In the progression to PCA, 738 up-regulated and 515 down-regulated genes were detected in samples. Analysis using Ingenuity Pathway Analysis (IPA) software revealed that 466 network and 423 functions-pathways eligible genes were up-regulated, and 363 network and 342 functions-pathways eligible genes were down-regulated. Up-regulated networks were identified around IL-1beta and insulin-like growth factor-1 (IGF-1) genes. The NFKB gene was centered around two up- and down-regulated networks. Up-regulated canonical pathways were assigned and four of them were evaluated in detail: acute phase response, hepatic fibrosis, actin cytoskeleton, and coagulation pathways. Axonal guidance signaling was the most significant down-regulated canonical pathway. Our data provide not only networks between the genes for understanding the biologic properties of PCA but also useful pathway maps for future understanding of disease and the construction of new therapeutic targets.

#### P06.075

#### Epigenetic biomarker for the early diagnosis of prostate cancer

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**Aim:** Prostate cancer is the commonest solid-organ malignancy diagnosed in men, and represents the second cause of cancer related death in men. In prostate cancer promoter hypermethylation of the glutathione-S-transferase P1 (GSTP1) is the most frequent DNA alteration.

In our study we want to investigate the potential use of the GSTP1 gene hypermethylation as a biomarker for the early detection of prostate cancer.

**Materials and methods:** For this study, we collected tissue and blood samples from 29 patients with histologically confirmed prostate adenocarcinoma and 24 patients with benign prostatic hyperplasia.

We performed methylation specific polymerase chain reaction (MSP) for the promoter region of GSTP1 on the collected samples.

**Results:** By methylation specific polymerase chain reaction GSTP1 promoter hypermethylation was not found in blood and tissue samples

from patients with benign prostatic hyperplasia, but it was found in 25 ( 86.2%) biological samples from patients with prostate adenocarcinoma.

Conclusions: Analysis by methylation specific PCR of GSTP1 promoter hypermethylation provides a specific tool for the early molecular diagnosis of prostate cancer in blood and tissue samples.

#### P06.076

#### Y chromosome haplogroup R1a is associated with prostate cancer risk among Macedonian males

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Prostate cancer (PC) is one of the most common male-specific cancers. Its incidence varies considerably between populations. Recent surveys suggest that PC is influenced by both genetic and environmental factors, although the etiology of the disease remains unknown in the majority of cases. Certain Y chromosomal lineages have been suggested to predispose individuals to prostate cancer in Japanese population, but no association has been found among Korean and Swedish patients. The aim of this study was to investigate the association between Y chromosomal haplogroups and predisposition to prostate cancer in Macedonian men. We studied 84 PC patients and 126 males from the general population of Macedonian ethnic origin. A total of 28 markers have been studied by multiplex PCR and SNAPSHOT analysis. Nineteen different Y haplogroups were determined; the most frequent being I1b-P37b, E3b1-M78, R1a-SRY 1532, R1b-P25 and J2b1a-M241. The frequency of R1a was significantly higher in PC patients (20.2%) in comparison with the controls (9.5%) [ $p=0.027$ ; OR=2.41 (1.09-5.36)]. When stratified according to age, even stronger association was observed between haplogroup R1a and prostate cancer in patients of  $\geq 65$  years of age [ $p=0.004$ ; OR=3.24 (1.41-7.46)]. Our results suggest that Y chromosome haplogroup R1a is associated with an increased prostate cancer risk in Macedonian men.

#### P06.077

#### Multifaceted preventive effects of single agent quercetin on a human prostate adenocarcinoma cell line (PC-3): Implications to nutritional transcriptomics and multi-target therapy

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The aim of the present study is to evaluate the effects of quercetin, a dietary flavonoid, on human prostate adenocarcinoma PC-3 cells. Lactate dehydrogenase (LDH) release, microculture tetrazolium test (MTT assay) and real-time PCR array were employed to assess the influences of quercetin on cell cytotoxicity, cell proliferation and expression of various genes in PC-3 cell line. Quercetin inhibited cell growth and proliferation and modulated the expression of genes involved in DNA repair, matrix degradation and tumor invasion, angiogenesis, apoptosis, cell cycle, metabolism and glycolysis. More importantly, quercetin inhibited the expression of genes responsible for progression from the androgen deprivation-responsive stage to the hormone deprivation refractory phase. In addition, no cytotoxicity of quercetin on PC-3 cells was observed. Taken together, as shown by the issues of the current study for the first time, the manifold inhibitory impacts of quercetin on PC-3 cells may introduce quercetin as an efficacious "magic shotgun" in order to be used in the future nutritional transcriptomic investigations and multi-target therapy to overcome the therapeutic impediments in crusade against prostate cancer.

#### P06.078

#### Low prevalence of PTEN mutations in a sample of Italian patients with Cowden or Cowden-like syndrome

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Cowden Syndrome (CS) is characterized by multiple hamartomatous and neoplastic lesions. 80-85% of CS patients are reported to carry detectable mutations in the PTEN gene. Recently, mutations in SDHB and SDHD genes have been described in some PTEN-negative CS patients. PTEN mutations have been also detected in about 60% of patients with Bannayan-Riley-Ruvalcaba Syndrome (BRRS), whose phenotype is partially overlapping that of CS.

We describe our preliminary experience of genetic testing in patients with features of CS or BRRS. Overall, we tested 15 patients: 4 fulfilled the criteria for the diagnosis of CS, 3 were affected by BRRS, whereas the remaining 8 had some criteria for CS and were therefore classified as CS-like.

Mutational analysis of PTEN was performed with automated direct sequencing using a multistep approach: first, exons 2 to 7 (containing 80% of the described mutations) and their flanking regions were analyzed. If no mutations were detected, the analysis was extended to exons 1, 8 and 9 and to the PTEN promoter region. PTEN-negative CS and CS-like patients were screened for mutations in the 4 coding exons of SDHD and the 8 coding exons of SDHB and flanking regions. Only in one CS patients a PTEN mutation was detected, which had not been reported before; no PTEN, SDHB or SDHD mutations were detected in the other CS, in CS-like and in BRRS patients. Based on these preliminary results, the genetic bases of CS and BRRS in our population appear to differ from those described in other populations.

#### P06.079

#### qPCR-HRM : a new approach in the screening of both point mutation and large rearrangement - application to oncogenetic

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In germline oncogenetic diseases, deleterious mutations, point mutations and large rearrangements are responsible of the inactivation in tumor suppressor genes as *BRCA1/BRCA2* in breast cancer or *MLH1/MSH2* in colorectal cancer. Until now, two different techniques were required to fully pre-screen those genes. We propose a new approach qPCR-HRM that combines quantitative PCR (qPCR) and high resolution melting curve analysis (HRM). This approach change also the way to perform high melting curve analysis. We illustrate this with our experience in the *MLH1* gene. 76 patients were fully pre-scanned for mutation in *MLH1* including 14 wild-type patients and 62 patients with known mutations (57 point mutations and 5 rearrangements). Moreover, a blind screening of 55 samples in triplicate was performed to assess sensitivity and sensibility in comparison to dHPLC-MLPA.

In the 131 patients, all the mutations detected by dHPLC+MLPA, either point mutations or rearrangements, were detected successfully with qPCR-HRM. The sensitivity was similar to dHPLC. However, the replicates in qPCR-HRM improve drastically the specificity. In the blind screening, without considering the triplicate, there were 170 false positives against 6 false positives with the triplicate (from a unique sample with obvious DNA quality problems).

With qPCR-HRM, pre-screening for point mutations and large rearrangements are realised in one tube and one step in a single machine without use of an automatic sequencer in the pre-screening process. The replicate approach increase the specificity of the HRM curve analysis. qPCR-HRM outperformed other techniques in term of rapidity and amount of data provided.

#### P06.080

#### Germline mutation in RAP80 impairs DNA damage response function

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Background: 5-10% of all breast cancers stem from hereditary predisposition to the disease. Mutations in two major susceptibility genes

*BRCA1* and *BRCA2* account for 20% of familial breast cancers. For the remaining 80%, genetic factors are largely unknown. Recently, a new *BRCA1*-interacting protein, RAP80, was identified. RAP80 plays an important role in *BRCA1*-mediated DNA damage responses by recruiting *BRCA1* to DNA double-strand breaks (DSB).

**Objective:** To investigate whether germline mutations in *RAP80* are associated with an increased risk of developing cancer.

**Methods:** Mutation screening was performed by CSGE and direct sequencing. For one of the observed alterations also functional studies were performed to assess influence on 1) Ubiquitin binding 2) RAP80 DSB localization 3) RAP80-BRCA1 complex DSB localization and 4) Genomic instability.

**Results:** Mutation screening from 112 index cases of Finnish breast cancer families revealed 10 alterations in *RAP80*, one of which was a novel exonic change. This novel alteration resulted in a dysfunctional protein product that displayed significantly reduced ubiquitin binding and double-strand break localization after DNA damage. It also impaired both *BRCA1* and *ABRA1* double-strand break recruitment, thus compromising *BRCA1*-mediated DNA damage response signaling. A significant increase in cytogenetically detectable chromosomal aberrations, particularly chromatid breaks, was also observed.

**Conclusions:** These results suggest that germline mutations in *RAP80* abrogate DNA damage response function and may be involved in genetic predisposition to cancer.

#### P06.081

#### Aberrant methylation of the genes *VHL*, *RASSF1*, *FHIT*, *SFRP1*, and *CDH1* in clear cell renal cancer

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Clear cell renal cancer (CCRC) is the most common tumor of the kidney, 200 thousands CCRC cases are registered worldwide annually. CCRC is characterized by tumor suppressor genes inactivation owing to several mechanisms including methylation. We have conducted the study of methylation of the genes *VHL*, *RASSF1*, *FHIT*, *SFRP1*, and *CDH1* in 123 CCRC for the development of renal cancer diagnostic and prognostic criteria. Methylation was detected by methylsensitive endonuclease *Bst*III digesting and following PCR, hypermethylated samples were confirmed using bisulphite sequencing. Aberrant methylation of *VHL* was observed in 14.6% (18/123), *RASSF1* - 53.7% (66/123), *FHIT* - 54.5% (67/123), *SFRP1* - 34.1% (42/123), and *CDH1* - 43.1% (53/123) cases. Methylation of at least one gene from these was detected in 85.4% (105/123) samples. *CDH1* methylation was associated with tumor invasion through the kidney capsule ( $P = 0.024$ ) and metastases in the regional lymph nodes and/or distant metastases ( $P = 0.001$ ). It was found that *RASSF1* was methylated more frequently in primary tumors with grade  $G_2$  than  $G_1$  ( $P = 0.047$ ). The genes studied could be used with some others tumor suppressors for formation of a diagnostic panel containing the genes frequently methylated in renal cancer, and aberrant methylation of the genes *CDH1* and *RASSF1* could indicate a primary tumor progression on different stages of CCRC.

#### P06.082

#### Identification of transcriptional targets by ChIP-Sequencing in t(X;1)-positive renal cell carcinomas

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Previously, we and others showed that in a subset of human renal cell carcinomas the bHLH-LZ transcription factor TFE3 is recurrently fused to a novel protein designated PRCC. Subsequently, we established that the resulting PRCCTFE3 fusion product acts as an oncogenic protein, both *in vitro* and *in vivo*. In addition, we found that PRCCTFE3 acts as a more potent transcriptional activator than wild-type TFE3. More recently, a functional cDNA screen revealed that TFE3 over-expression renders cells insensitive to the anti-proliferative effects of the G1/S cell cycle regulator pRB. We propose that also the PRCCTFE3

fusion protein may act through a cell cycle-mediated deregulation of proliferation. In order to identify downstream transcriptional targets of the PRCCTFE3 fusion protein, we initiated to use chromatin immunoprecipitation (ChIP). Specifically, we are employing a recently developed variant of this technology, called ChIP-Sequencing, which combines ChIP with massive parallel sequencing to identify and quantify *in vivo* protein-DNA interactions on a genome-wide scale. The identification of novel PRCCTFE3 transcriptional targets and its implications for our understanding of the role of cell cycle (de-) regulation in renal tumor development will be discussed.

#### P06.083

#### The tyrosine kinase RET interacts *in vivo* and *in vitro* with AIP

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RET is a tyrosine kinase transmembrane receptor expressed in two main alternative isoforms: RET9 and RET51. RET transduces a positive signal leading to survival, differentiation or migration in the presence of its ligand GDNF, whilst in absence of the ligand, RET is cleaved generating a proapoptotic fragment which initiates a signalling pathway for apoptosis. Hitherto, signal transduction leading to apoptosis is still unclear.

We performed a screening to identify the interacting proteins of the long isoform of RET, using a modified two-hybrid yeast complementation assay, the split-ubiquitin system against a human brain expression library.

One of the proteins we identified with this method was the aryl hydrocarbon receptor interacting protein (AIP), a tumor-suppressor protein recently found mutated in pituitary adenoma. We showed that RET-AIP interaction was maintained both in cell lines of different origin (human embryonic kidney and neuroblastoma) and in pituitary gland *in vivo*. In addition, we identified the pro-apoptotic domain of RET as responsible for AIP interaction, regardless of the presence of pituitary adenoma specific mutations. AIP and RET genes were sequenced in 28 pituitary adenoma but no relevant mutation has been found. Finally, we showed that AIP-RET interaction does not require RET kinase activity or kinase dependent signal transduction and it prevents the formation of AIP-survivin complex. The identification of AIP-RET complex represents a starting point for studying key cellular processes involved in RET induced apoptosis.

#### P06.084

#### MS-MLPA to study the contribution of epigenetic silencing in Retinoblastoma.

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Recent studies in the field of DNA methylation have lead to the awareness that epigenetic changes may represent an alternative or complementary mechanism to mutational events in tumour progression. In particular methylation of CpG islands in the promoter regions of a large number of tumour suppressor genes is observed in several human cancers. Previous studies on Retinoblastoma (RB) tissues showed frequent hypermethylation of the DNA-repair genes *MGMT* and *MLH1* and the tumor suppressor gene *RASSF1A*. Methylation-specific MLPA (MS-MLPA) has been recently described as a method that allows the simultaneous identification of epigenetic changes at multiple sites. We applied this technique to study epigenetic changes in 10 RB samples and we compared results to those obtained in normal retina. Tumour tissues showed frequent hypermethylation of *MGMT* (70%), *MSH6* (60%), *CD44* (50%), *PAX5* (50%) and *GATA5* (30%). Since these genes are involved in DNA repair (*MSH6*), cellular differentia-

tion (*PAX5* and *GATA5*), and cell-to-cell communication (*CD44*), their epigenetic silencing could play an important role in RB initiation and progression. Therefore this study not only confirms the importance of *MGMT* inactivation, but also identifies new interesting candidate genes for RB. Aberrant methylation of these factors could play a key role in tumour development especially in bilateral cases, where chromosomal imbalances are less frequently observed.

#### P06.085

##### An *Sdh* knockout mouse shows no evidence of paraganglioma or pheochromocytoma tumor development but does exhibit peripheral ventilatory insufficiency

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*SDHD* is a human tumor suppressor gene and a subunit of succinate dehydrogenase, a component of both the TCA cycle and the electron transport chain.

Here we describe a mouse KO of *Sdh* as a model for pheochromocytoma/paraganglioma with 30 month-follow up, and as a model for carotid body-mediated peripheral ventilatory insufficiency.

The absence of live homozygote (-/-) offspring indicates that complete loss of *Sdh* results in embryonic lethality. Knockout of the *Sdh* gene did not lead to tumor development at any stage of the normal lifespan of these mice, in contrast to the highly penetrant phenotype in humans. A single *Sdh* +/- mouse showed unilateral 5-fold hyperplasia of the carotid body.

*Sdh* +/- mice showed no gross physical abnormalities, similar body and organ weights to wildtype mice, and no genotype-related pathology. The ultrastructure of the carotid body and adrenal mitochondria was normal.

Breathing in small animals is regulated by central chemoreceptors located in the ventral medulla, and the peripheral chemoreceptors in the carotid bodies.

The carotid body is essential to the peripheral ventilatory response, and loss of SDHD may affect carotid body function. We found that the carotid body-related ventilatory response, the so-called peripheral response, is indeed compromised. We show that while the central CO<sub>2</sub>-driven ventilatory response was intact in both wildtype and *Sdh* +/- mice, the CO<sub>2</sub>-driven peripheral ventilatory response was severely compromised in *Sdh* +/- mice.

#### P06.086

##### Differential repair of UVA versus UVB-induced cyclobutane pyrimidine dimers in the genome of human keratinocytes

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Worldwide, one in three cancers is skin-related and the WHO expects the skin-cancer epidemic to increase. Solar ultraviolet radiation (UVR) by inducing DNA photo-lesions, has become the prime cause of most skin cancers. These cancers could be prevented if we protect ourselves from UVR.

UVR comprises three main regions: UVC is absorbed by the ozone layer and does not affect the skin. UVB is directly absorbed by DNA and induces different forms of lesions like cyclobutane pyrimidine dimers (CPDs). In contrast with UVB, UVA is indirectly absorbed by DNA. UVA is suspected to play a key role in induction of skin tumors and may be even more important than UVB in mutagenesis. CPDs have been found to be induced in human skin cells exposed to UVA through a different mechanism but the mechanism of CPD induction by UVA is not clearly identified. Most sunscreens filter out UVB absorption, but they cannot block most of UVA, so they do not help to prevent skin cancer.

A better assessment of the routes by which UVA and UVB induce CPDs in DNA, may lead to prevention of skin cancer. For this purpose in the present study by use of the highly sensitive single cell gel electrophoresis (comet assay) formation and repair rate of UVA-induced CPDs versus UVB-induced CPDs was compared.

Despite seeing a 10-fold greater dose of UVA, UVB induction of CPD was significantly greater. However, the rate of UVA-induced CPD repair appeared to be faster than the rate of UVB-induced CPD repair.

#### P06.087

##### Diverse functions of Slug, a master regulator of EMT, in prostate cancer cell lines

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The transcription factor Slug (SNAI2) is capable of mediating the epithelial-mesenchymal transition (EMT), which is thought to be a crucial step in metastasis. There is moreover increasing evidence that this factor has additional functions in tumor progression. We have investigated the effects of siRNA-mediated down-regulation (knockdown) of Slug in prostate cancer cell lines.

Among the prostate carcinoma cell lines, PC-3 and PC3-16 subline (kindly provided by the Homburg DPKK group) show the moderate and highest expression of *Slug*. Therefore, Slug knockdown was performed in PC-3 and PC3-16 cells. In each line, efficient down-regulation at the mRNA and protein level was achieved. Long-term knock-down of *Slug* expression induced a severe decline in cell proliferation in both cell lines, with a prominent G0/G1 arrest in PC3-16 cells. Apoptosis was slightly enhanced in PC-3 cells only. SNAI2 siRNA-treated cells did not tolerate detachment from the culture plates, probably due to down-regulation of integrin  $\alpha 6\beta 4$ . Moreover, suppression of *Slug* expression strongly abolished invasiveness of PC-3 cells. Finally, knock-down of *Slug* expression disturbed both the microtubule and actin cytoskeletons resulting in grossly enlarged cells with a jellyfish-like phenotype. Together, these data suggest that the most pertinent function of *Slug* in the prostate cancers that express it, is to act as a regulator of cell adhesion and cell shape maintenance.

#### P06.088

##### The association of 5a-Reductase type 1 with HBV positive hepatocellular carcinoma (HCC) risk in male Chinese

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Introduction: Epidemiological studies suggested that male predominance of hepatitis B positive hepatocellular carcinoma was associated with serum levels of androgens. Although androgen levels are believed to be influenced by genetic factors, there is no definitive information to indicate which genetic variations in the androgen metabolic pathway are associated with the risk of hepatocellular carcinoma (HCC) development.

Aim: The aim of the present study was to study polymorphisms in SRD5A1, a gene related to androgen metabolic pathway in converting testosterone to its more potent biological form, dehydrotestosterone (DHT), and their association with HCC.

Methods: 300 HBV positive HCC male patients and 2000 male Chinese population controls were recruited in this study. Using the International HapMap Phase II data on the Han Chinese (CHB) cohort, 3 tagging SNPs (rs248807, rs11738248, rs824811) were selected by spectral analysis. Genotyping of these three SNPs was performed using Allele Specific Tm-shift PCR method.

Results and conclusion: The genotype frequencies of rs248807, rs11738248, rs824811 followed Hardy Weinberg equilibrium ( $p>0.05$ ). The SNP rs11738248 was significantly associated with HCC ( $X^2=10.069$ ,  $P=0.007$ ) while the association for SNP rs248807 was modest. However, the SNP rs824811 was not in association with HCC ( $X^2=0.54$ ,  $P=0.763$ ). All the result suggests that SRD5A1 gene may associate with the development of HCC in men. We previously showed that SRD5A2 isoenzyme was associated with HCC. The results on another isoenzyme SRD5A1 presented here further confirmed the role of androgen metabolites in the carcinogenesis of HBV related HCC.

**P06.089****Molecular genetic investigation in patients with lung cancer: parallel analysis of STRs in cell-free DNA and SNP polymorphisms within 15q24-q25 region in genomic DNA**

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**P06.090****Mutations of Succinate Dehydrogenase and Fumarate Hydratase. A TCA Cycle Gene Mutation Database Update**

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Two enzymes of the tricarboxylic acid (TCA) cycle, fumarate hydratase (FH) and succinate dehydrogenase (SDH), involved in fundamental processes of energy production, are now known to be tumor suppressors.

While deficiencies of FH and SDH(A) result in severe early-onset encephalopathy, germline mutations of SDH genes are a major cause of hereditary paraganglioma and pheochromocytoma, and FH mutations result in the hereditary leiomyomatosis and renal cell cancer (HLRCC) tumor syndrome.

The SDH mutation database (DB) was launched in 2005 and with the inclusion of FH is now the TCA Cycle Gene Mutation Database. The TCAC DB is gene-centered and based on LOVD, with each gene having a separate summary page listing general information and providing access to tables containing variant information and various search options. The HGVS conform nomenclature provides an accessible resource easing the description of new mutations.

371 unique variants are currently described in the database. We describe the current status of each gene and recent developments.

In 2008 the database attracted >75,000 page hits, a doubling compared to 2007, and saw an average monthly use by 377 unique IP addresses. The increasing community use of the TCAC DB indicates widespread acceptance and increasing utility.

The TCAC DB represents a valuable resource for clinicians, clinical geneticists, and researchers interested in paraganglioma/ pheochromocytoma and HLRCC.

**P06.091****Anti-MUC1 VH can redirect chimeric antigen receptor (CAR) cytotoxic effector function**

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Chimeric antigen T cell receptors provide a good approach for adoptive immunotherapy of cancer, especially in the context of cancerous cells that fail to express major histocompatibility complex antigen and co-stimulatory molecules. Clinical applications of these receptors are limited, mostly due to xenogenic origin of the antibodies which cause immunogenic reactions. VH are the smallest fragments of antibodies that have great homology to human VH and low immunogenic potential. MUC1 is a highly attractive immunotherapeutic target owing to increased expression, altered glycosylation, and loss of polarity in more than 80% of human malignancies. We used anti-MUC1 VH as an antigen binding domain, CD28 and CD3ζ as signaling domains and IgG3 as a spacer in a chimeric receptor construct. This construct was transfected to Jurkat cells. The transfected Jurkat cells were exposed to MUC1 positive MCF7 cells. Then we analyzed the secretion of IL2, proliferation of Jurkat cells and death of MCF7 cells. These data revealed that the VH chimeric receptor can target tumor associated antigen positive cells. Regarding the efficient and specific function of VH chimeric receptor and non immunogenic nature of VH, these chimeric receptors might be used as promising candidates for clinical applications.

**P06.092****Molecular Analysis of T-Cell Receptor Gene Rearrangements**

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The evaluation of clonality on the T cell receptor locus can be an important tool in detecting T-cell non-Hodgkin lymphoproliferations. The locus is comprised of a 160 kb region on Chromosome 7 and has well defined variable (V), joining (J) and constant (C) gene segments. Molecular interrogation of the rearrangements in the variable and joining segments can be used to compare fragment sizes of DNA from a normal polyclonal T-cell population (many different sizes) to DNA from a clonal T cell population with suspected lymphoproliferations (few different sizes). The comparison can be accomplished with PCR and subsequent fragment size analysis via capillary electrophoresis. PCR methods using fluorescent labeled primers designed to anneal to the flanking, conserved, regions of interest on the locus have been established. We describe new methods used to accomplish the DNA comparison using Capillary Electrophoresis (CE) systems that produce consistent results. Use of these CE systems could greatly benefit an individual or association of researchers with multiple instruments in many locations. Traditionally, conclusions were drawn by comparing the DNA fragments by visual inspection. We demonstrate new software analysis methods that allow the scientist to make quantitative conclusions.

**P06.093****Presence of activating KRAS mutations correlates significantly with expression of tumour suppressor genes DCN and TPM1 in colorectal cancer**

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Despite the identification of the major genes and pathways involved in the development of colorectal cancer (CRC), it has become obvious that several steps in these pathways might be bypassed by other as yet unknown genetic events that lead towards CRC. To improve our understanding of the genetic mechanisms of CRC development, we used microarrays to identify novel genes involved in the development of CRC. Using real-time PCR, we also searched for chromosomal abnormalities within candidate genes and the expression pattern in the case of KRAS mutation. We detected significant previously unde-

scribed underexpression in CRC for genes SLC26A3, TPM1 and DCN, with a suggested tumour suppressor role. We also describe the correlation between TPM1 and DCN expression and the presence of KRAS mutations in CRC. When searching for chromosomal abnormalities, we found deletion of the TPM1 gene in one case of CRC, but no deletions of DCN and SLC26A3 were found. We are the first to describe underexpression of three important tumour suppressor genes in cases of CRC, thus implicating them in the development of this type of cancer. Moreover, we found underexpression of the TPM1 gene in a case of CRCs without KRAS mutations, showing that TPM1 might serve as an alternative path of development of CRC. On the other hand, the correlation of DCN underexpression with the presence of KRAS mutations suggests that DCN expression is affected by the presence of activating KRAS mutations, lowering the amount of the important tumour suppressor protein decorin.

#### P06.094

##### Trefoil Factor (TFF) genetic variation in gastric carcinogenesis

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The genes encoding the trefoil factor peptides (*TFF1*, *TFF2* and *TFF3*) are clustered in a 55 kb region on 21q22.3. TFFs are a small protein family secreted onto the mucous epithelia that play an important role in gastrointestinal mucosal maintenance. We have examined whether genetic variation in these genes is associated with gastric carcinogenesis. We genotyped five potentially functional SNPs (rs2156310 in *TFF1* 5'UTR, rs13052596 in *TFF2* promoter, rs225334 and rs4920084 in *TFF2* 3'UTR, and rs11701143 (T36A) in *TFF3*) and 1 novel insertion polymorphism (c.\*68\_69InsCTT, in the 3'UTR region of *TFF2*) in 476 patients with gastric preneoplastic lesions followed a mean of 14 years. The Correa's Index was used to evaluate the progression, regression or stability of the lesions. Two polymorphisms (rs225334 and c.\*68\_69InsCTT) were also genotyped in 217 cases of gastric cancer (GC) and in 891 controls from the EPIC cohort. Heterozygote genotypes for rs11701143 (c.106AG) and for *TFF2* c.\*68\_69InsCTT showed association with lesion progression (OR:2.12, p=0.04 and OR:2.44, p=0.049, respectively) when compared to common homozygotes. Due to their low MAF (0.03 and 0.05, respectively), variant homozygotes were not observed. SNP rs2156310 (c.-2C>T) was also associated with lesion progression in the dominant model (OR:1.8, p=0.024). Regarding association with GC we only found a slightly significant association between heterozygosity for rs225334 and the diffuse type of GC (OR:0.55, CI:0.3-0.9, p=0.047). Although replication and functional studies are needed, these results suggest that variations in *TFF* may have a role in gastric carcinogenesis.

#### P06.095

##### Down-regulation of Tristetraprolin, a negative regulator of mRNA stability, in breast carcinogenesis

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Post-transcriptional regulation plays a central role in cell differentiation and proliferation. The functional relevance of this process is highlighted by pathologies such as chronic inflammation and cancer, wherein occurrence tightly correlates with a dysregulation in mRNA stability. Nonetheless only a handful of the regulatory factors involved in this mechanism have been identified. Among these, Tristetraprolin (TTP) is the prototype of a family of RNA-binding proteins with unusual zinc-finger binding domains that binds to the AU-rich sequences in the 3'UTR of the genes promoting their physiological decay. VEGF and IL-8, two main cytokines involved in angiogenesis and metastasis, are specifically regulated by TTP. Here we investigated if TTP could correlate with tumor aggressiveness in breast cancer and if it may represent a novel prognostic factor for this neoplasia. By quantitative PCR and Western blot analysis we determined the amount of TTP in different

breast cancer cell lines, finding a low expression in two cell lines characterized by high aggressiveness and metastatic potential. In these cells loss of TTP expression correlates with increased IL-8 secretion, probably as a result of pathologically stable mRNAs. By sequence analysis of the gene, we identified some genetic polymorphisms which may be associated with TTP disappearance and performed both functional analysis and association studies in a sample of French patients affected by breast cancer.

Our data underline the importance of tristetraprolin in breast carcinogenesis and show that tumor progression can be achieved by interfering with physiological mRNA turnover.

#### P06.096

##### Influence of *TYMS* expression and genotype on the clinical outcome of colorectal cancer patients treated with 5-Fluorouracil.

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Thymidylate synthase (TS) expression levels seem to be related with clinical outcome and response to 5-FU chemotherapy. Three polymorphisms have been proposed as modulators of TS mRNA transcriptional and translational efficiency: a tandemly repeated sequence (2R/3R) in the 5' UTR, a SNP within the 3R allele and a 6 bp deletion in the 3' UTR.

Our aim was to evaluate the influence of *TYMS* expression and genotype on the clinical outcome of patients treated with 5-FU.

We have analysed expression levels in healthy and tumour tissues, the entire coding sequence in DNA from colonic mucosa, and the 5' and 3' UTR in healthy and tumour tissues from 64 CRC patients.

A statistically significant correlation was observed between tumor TS expression levels and clinical outcome: low TS mRNA levels were associated with longer disease-free-survival ( $P=0.030$ ) and longer overall survival ( $P=0.048$ ), while no significant difference in TS gene expression was observed between 2R and 3R genotypes. Linkage disequilibrium between the 2R/3R and the 6 bp 3' UTR polymorphisms was detected, with a significant association between the 3RG allele and the 6 bp 3' UTR deletion allele.

These results are in agreement with the growing evidence that the control of TS may require multiple mechanisms acting in close coordination with one another and with the suggestion that *TYMS* genotyping alone cannot predict response to 5-FU.

#### P06.097

##### Pharmacogenomic effects of UDP-glucuronosyltransferase 1A1 on irinotecan-induced drug reaction and serum bilirubin levels.

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Irinotecan is metabolized to active form which is further conjugated and detoxified by the UGT1A1 enzyme. The severe toxicities in patients who receive irinotecan are related to its genetic variants, and the serum total bilirubin (BIL) levels are useful for the prediction of adverse reactions to irinotecan. However, the relationship between the specific UGT1A1 genotype and BIL levels has not yet been confirmed. Then, we studied 443 cases with no history of liver dysfunction to explore their relationship. Genomic DNAs were extracted from peripheral leukocytes after the informed consent was obtained. The assays for genotyping the polymorphisms in the UGT1A1 (\*6, \*27, \*28, \*60) were based on either Invader assay or direct sequencing. The frequencies of \*28 and \*60 variants were much lower than those published results for whites, while the frequencies of \*6 and \*27 variants were much higher. Furthermore, 89% (24/27) subjects with hyperbilirubinemia had \*6, \*28 or \*60 variants. This study showed that several UGT1A1

genotypes were significantly associated with the increased BIL levels. These findings will be useful for further pharmacogenomical studies on adverse reactions to irinotecan.

#### P06.098

#### Uterine leiomyoma with high proliferative index versus leiomyosarcoma: analyzes of allelic imbalance

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Uterine leiomyosarcoma (ULMS) is rare and highly malignant smooth muscle tumor. Differential diagnosis between uterine leiomyoma with high proliferative index (ULM) and ULMS is one of the basic problems in pathology for nowadays. The singular morphological differential criteria is the mitosis index (quantity of mitoses).

We investigated allelic imbalance (AI) to find out genetic differences between ULM and ULMS. Microsatellite analysis was evaluated by PCR using 4 polymorphic markers for chromosomal regions 3p14, 10q22, 10q23, 10q26 in 14 patients with 23 formalin-fixed paraffin-embedded samples (15 ULMS, 5 ULM, 3 metastatic lymphatic nodules). 10 leiomyoma specimens from patients with benign process were suggested as control.

Our results demonstrated AI in ULMS samples with following frequencies: D3S1295 - 5/14 (35.7%), D10S218 - 6/14 (43%), D10S541 - 6/14 (42.9%), D10S1213 - 5/14 (35.7%). Occurrence of investigated genetic alterations (at least for the one of microsatellite markers) was revealed in 13 leiomyosarcomas (91%). The AI frequency in ULM samples was found only for one patient..

This research show that AI analysis may be helpful for accurate exclusion between ULMS and ULM though further investigation is needed. Increasing of molecular marker number will be necessary for diagnostically challenging cases of uterine smooth muscle tumors.

#### P06.099

#### Status of some tumor-suppressor loci in uveal melanoma

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Purpose. We investigated a panel of uveal melanomas (UM) for the presence of allelic losses at some chromosomal regions where structural abnormalities had previously been found, and the methylation status of tumor suppressor genes supposed to be involved in UM pathogenesis.

Methods. Loss of heterozygosity (LOH) at chromosomal regions 1p36, 1p31.3, 3p25.3 (VHL), 3p21.3 (RASSF1A), 3p14.2 (FHIT), 3q26.3 (TNFSF10), 9(p21.2-p21.3) (CDKN2A), 10(q23.2-q23.3) (PTEN), 13q14.2 (RB1) was investigated by PCR-based microsatellite analysis in 107 uveal melanomas. Samples were also analyzed for the methylation status of VHL, RASSF1A, FHIT, CDKN2A and RB1 by methylation-sensitive restriction enzyme PCR. Clinical and histopathological parameters were analyzed together with genetic abnormalities.

Results. Monosomy 3 was detected in 48 of 107 tumors, and showed a significant association with the presence of epithelioid cells ( $P<0.0001$ ) and ciliary body involvement ( $P=0.001$ ). Methylation analysis discovered frequent methylation of RASSF1A (26 patients, 24%), predominantly in UM without monosomy 3. LOH at all 1p markers was found in 25 samples and a statistically significant association with extrascleral invasion of the tumor was determined ( $P=0.01$ ). We detected infrequent hypermethylation of CDKN2A and LOH at 9p21.2.

Conclusions. We suggest that deregulation of the RASSF1A may be involved in tumorigenesis in a significant proportion of uveal melanomas. We point out an association of large-scale 1p deletion with extrascleral invasion of tumor, which is found to be independent of monosomy 3. Inactivation of CDKN2A and RB1 with promoter methylation or LOH is not the major mechanism of the pathogenesis in UM.

#### P06.100

#### HSPC300 modifies clear renal cell cancer (RCC) risk in complete VHL gene deletion cases

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Gross VHL gene deletions are relatively common in Eastern Baltic Sea region in von Hippel-Lindau (VHL) syndrome patients, comprising around 40% of all mutations. There is well documented genotype-phenotype correlation between particular missense mutations and risk of pheochromocytoma, as well as truncating mutations and clear renal cell carcinomas (RCC). Paradoxically, complete VHL gene deletions were thought to be associated with relatively mild phenotype, suggesting low risk of developing RCC in complete VHL gene deleted patients.

Comprehensive VHL genetic testing service and patients surveillance in Lithuania is focused in Hematology, Oncology and Transfusion medicine center, Vilnius university hospital Santariskiu clinics. VHL gene dosage evaluation by real-time PCR followed by direct sequencing for normal gene dose cases is preferred approach.

We report 3 patients with complete VHL gene deletion and delineate clinical phenotype. Unusual severe phenotype with cerebral hemangioblastomas, RCC and multiple renal/pancreatic cysts was observed, comprising to our knowledge the first RCC case in a patient with whole VHL gene deletion. Additional MLPA and specific multiplex PCR tests revealed intact neighbouring actin regulator HSPC300 gene in this family. Our results are in agreement with increasing evidence that retention of HSPC300 gene is responsible for increased RCC risk in gross VHL gene deleted cases. Our findings suggest whole VHL gene deletion carriers with retention of HSPC300 have the same probability to develop RCC as carriers with partial VHL deletion. HSPC300 testing in gross VHL gene deletion cases is useful approach for the individualisation of the clinical management for these patients.

#### P06.101

#### Genetic profiling of Loss of Heterozygosity in Pediatric Tumors

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Loss of heterozygosity (LOH) analysis is useful for research regarding the study of pediatric tumors such as Wilms tumor, hepatoblastoma, and neuroblastoma. LOH is the loss of the remaining functional allele of a gene by genetic or epigenetic changes when the other allele is already inactivated, usually the result of an inherited germ-line mutation. Tumor-suppressor genes encode proteins critical to protecting cells from cancer. LOH of these genes is a key event in tumorigenesis. To investigate LOH, specific assays have been developed involving PCR and capillary electrophoresis. The regions of interest are amplified using one fluorescently-labeled and one unlabeled PCR primer for each locus interrogated. Two types of samples are analyzed: genomic DNA isolated from normal cells, and genomic DNA isolated from tumor cells, both from the same individual. We present a new capillary electrophoresis system that can be used for performing research involving routine relative fluorescent quantitation assays. LOH analysis is demonstrated by analyzing a variety of pediatric tumor samples. We describe the methods used to perform the DNA comparison on a capillary electrophoresis platform suitable for research in validated environments. This methodology would also be useful to a single researcher or a consortium of investigators with multiple instruments who require consistent and comparable results from each instrument.

#### P06.102

#### Identification of transcription factor ZIC2 as a novel component of B-catenin/TCF4 complex

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The Wnt/B-catenin pathway plays a critical role in proliferation and differentiation of epithelial cells of the intestinal mucosa. ZIC2, a member

of the ZIC gene family, encodes a zinc finger transcription factor which is important in neural crest proliferation and differentiation. We identified ZIC2 as highly expressed gene in subset of colorectal tumors using microarray validated by QPCR and immunohistochemistry. The expression of ZIC2 was consistent with methylation status of gene promoter. Here we show that ZIC2 interacts with  $\beta$ -catenin/TCF4-mediated transcriptional activation of the  $\beta$ -catenin through binding to C terminal domain of TCF4. The zinc finger domain of ZIC2 was found essential for this interaction. By using animal cap study on Xenopus, we show that Zic2 abrogates the effect of  $\beta$ -catenin on known Wnt target genes, siamois and Xnr3. Moreover, ZIC2 represses the expression of Wnt target genes cyclinD1 and c-Myc. Cell cycle analysis by flow cytometry showed an increased fraction of cells in the G1 phase and subsequent decrease in S phase in ZIC2 transfected cells. This is the first report of a role for ZIC2 as a repressor of TCF4/B-catenin complex.

#### P06.103

##### Cytogenetic studies on Breast cancer patients in Tamilnadu, India.

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Breast cancer is the second most common cancer among women in India. The focal aim of the present study has been indented to analyze the chromosomal alterations and micronucleus in different stage of BC patients in Tamilnadu population. The present study also aims to investigate whether the CA15.3 level with BC patients exhibit increased CA to address this issue. Furthermore, the most of samples were recruited on the basis of family history and identify the genetic defects on BC patients. In the present study 63 experimental subjects were selected on the basis of CA15.3 marker which is the most widely used serum biochemical tumor marker in breast cancer and equal number of controls were selected and confirmed by CA53 level.

In the present study statistically significant results were obtained in experimentals compared to controls. In the present study deletion and translocation were frequently observed in chromosome 1, 11 and 17. (46, XX, del (1p<sup>-</sup>); 46, XX, del (11q<sup>-</sup>); 46, XX, del (17q<sup>-</sup>)). 46, XX, t (1q<sup>+</sup>16q<sup>-</sup>) ; 46, XX, t (11q<sup>+</sup>7p<sup>-</sup>) ; 46, XX, t (17q<sup>+</sup>4q<sup>-</sup>)). Inversion and satellite formation (21s<sup>+</sup>) also frequently observed in experimentals. In MN frequency also showed higher degree in experimentals compared to controls ( $P<0.001$ ).

In the near future, we can look forward to the identification of novel breast cancer predisposing genes due to rapid advancement of gene discovery technologies.

The Identification and functional characterization of such genes will have a significant impact on breast cancer research and early detection.

#### P06.104

##### Evaluation of Autoantibody-Serum-Biomarkers for Breast Cancer Screening.

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Tumor -autoantibodies in patient's sera are biomarker- candidates for minimal invasive screening and diagnosis of autoimmune- as well as cancerous disease. Using serum samples from breast cancer patients and controls we performed a candidate marker screen on nitrocellulose membranes containing 38000 human proteins derived from a fetal-brain expression library. From the initial screen we identified 642 candidate antigens reactive with serum-antibodies from patients and controls. The respective e. Coli -clones were used to express recombinant proteins and produce targeted protein-microarrays. We set up methods for high throughput recombinant protein expression and production of protein microarrays. Using these protein-chips we optimized conditions for suitable detection of serum-auto-antibody profiles using only a few micro-liters of patient's serum.

Analysing a test-set comprising sera from benign (n=16) and malign breast nodules (n=24) and from normal controls without any breast nodule (n=20), we could delineate an antigen profile which enabled good distinction between patients with and without nodular disease. However distinction between malign and benign tumors could not be achieved.

#### P06.105

##### Bilateral breast cancer: experience of an out-patient cancer genetics clinic in the United States

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These past two years, over 300 patients with breast cancer have undergone risk assessment and genetic counseling in our cancer genetics clinic, held in the outpatient cancer center of a major private hospital. Over half have undergone BRCA 1-2 gene analysis; insurance restrictions often preclude additional testing. Thirteen women with bilateral breast cancer were among these patients referred. We reviewed clinical, histological, genetic and demographic characteristics of these women, hypothesizing that those with BRCA mutations might differ from those in whom testing was negative. It was expected, based on risk calculation software, that 20-40% of patients would have BRCA mutations.

Nine women had metachronous breast cancer, four were synchronous. Average age at first diagnosis was 42 (range 31-74 years); for the nine with metachronous cancers 1 to 20 years intervened between cancers. For 22/26 malignancies the specific histological type was available, and for 18/26 the estrogen receptor status was known. All 22 of these were ductal carcinomas; two women had bilateral ductal carcinoma *in situ*, two others had one invasive and one *in situ* malignancy; all others were bilateral and infiltrating. Estrogen receptors were positive in 18 cancers, negative in 4. All women had BRCA 1-2 testing; one had a truncating mutation in BRCA1, one a truncating mutation in BRCA2 and a third a missense mutation of unknown significance in BRCA1. CHEK2 gene sequencing was negative in two. Thus, our hypotheses about proportion of BRCA carriers and differing histological characteristics were not confirmed in this small group of patients.

#### P06.106

##### The prevalence of mutations in BRCA and NAIP genes in two common malignancies in Romanian population

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Mutations in BRCA1 and 2 genes confer a substantial lifetime risk to breast and colorectal cancer. The disease risk may be increased by mutations in other genes involved in control of apoptosis (e.g. NAIP). The spectrum of mutation in these genes has been not previously investigated in Romanian patients with breast or colon cancers.

Aim. To evaluate the contribution of BRCA and NAIP mutations in patients with breast or colorectal cancer

Material and methods. We started this study in 2007 by selecting Caucasian patients with familial breast (n=70 women) or colorectal cancer (n=70) from two medical centers from Bucharest. Cancer diagnostics were confirmed by clinical and paraclinical approach. The presence of BRCA1 185 AG, BRCA1 5382 insC, BRCA2 6174 delT and NAIP del exon 5 mutations were assessed using commercial kit and classical PCR. In addition, the presence of other mutations in several regions from these genes using indirect methods (SSCP and HRM) was tested.

Results. We identified the heterozygous BRCA1 5382 insC mutation in three women with breast cancer using both commercial kits and classical PCR- RFLP based tests. For other three patients with breast cancer abnormal pattern of migration in SSCP and HRM method was identified. We also found no mutations in BRCA genes in patients with colorectal cancer.

Conclusion. Our preliminary results showed that BRCA1 5382 insC could be the most common mutation in women with familial breast cancer. The homozygous NAIP deletion is not present in our lots. (Project Romania PNII- 42161)

**P06.107****Large BRCA1 genomic rearrangements in Czech high-risk breast-ovarian cancer families**

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BRCA1 and BRCA2 germline mutations predispose to breast and ovarian cancer. In addition to point mutations, small insertions and deletions there are also large genomic rearrangements (LGRs) in BRCA1/2 genes. For detection of these LGRs we used the multiplex ligation-dependent probe amplification (MLPA). We have screened for LGRs about 1100 unrelated patients with familial breast and/or ovarian cancer in whom a deleterious mutation in BRCA1 and BRCA2 was not detected. Characterization of the LGRs was carried out by performing long-range PCR followed by sequencing.

We identified 34 (3.1%) patients with 11 different LGRs (all of them in BRCA1 gene), including a complete deletion of BRCA1 gene. Existence of this entire BRCA1 gene deletion was proved by using two alternative MLPA kits (SALSA P002B and P087) that have different localization of ligation probes. In addition - during High Resolution Melting analysis of all BRCA1 gene exons of this patient none polymorphism has been detected what shows an evidence of loss of heterozygosity. LGRs make a significant contribution to the whole amount of disease causing mutations. In our opinion detection of LGRs in BRCA1 gene should be a part of routine screening. No LGRs in BRCA2 gene were detected yet.

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**P06.108****High risk BRCA1 and BRCA2 alleles in Estonia; family with a rare BRCA2 mutation**

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We have lately established a hereditary breast and ovarian cancer counselling and genetic testing service. The testing is two-phase. At first, cost-efficient arrayed primer extension (APEX) chip is used for mutation screening of referring persons with personal or family history of breast and ovarian cancer. If no mutations are found, full sequencing of BRCA1 and BRCA2 genes is performed in high-risk patients.

By February 2009, 62 persons had been counselled and tested in the Dept. of Genetics, Tartu University Hospital, Estonia. 12 (19.4%) had high-risk alleles present in BRCA1 and BRCA2 genes. From nine high-risk alleles known to date in Estonia, five were found: c.300T>G (p.C61G), c.1186delA, c.4154delA, c.5382insC in BRCA1 gene and c.9168insA in BRCA2 gene.

We would introduce a family with rare c.9168insA BRCA2 mutation. According to Breast Cancer Information Core database, this has been formerly reported only once. A woman with breast cancer diagnosed at age 66 was counselled. Her sister had got the same diagnosis at 40 years age and ovarian cancer at 45 years; she had died at 50. Their father had fell ill with breast cancer at 87. The father and his daughter had both the c.9168insA BRCA2 mutation. The daughters' three descendants were examined and one of them was found to be the mutation carrier. The family is ethnic Russian, originating from Moscow Oblast.

Genetic services are essential for efficient management of hereditary cancer. Sequencing results will be further used for updating the chip according to local profile of risk alleles.

**P06.109****Screening for BRCA1/2 gene large rearrangements in 260 Spanish hereditary breast cancer cases: high occurrence of deletions in the BRCA2 gene in male breast cancer patients**

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*BRCA1 and BRCA2 germ-line mutations predispose to breast and ovarian cancer. The multiplex ligation-dependent probe amplification (MLPA) is a method for detecting gross deletions or duplications of DNA sequences, aberrations which are commonly overlooked by standard diagnostic analysis.*

*To determine the incidence of large rearrangements in cancer predisposition genes BRCA1 and BRCA2 we have analyzed both genes in 260 individuals from hereditary breast/ovarian cancer families without deleterious point mutations.*

*A total of 8 pathogenic rearrangements in the BRCA genes were found, accounting for 3.1% of the cases. In two patients from families with breast and ovarian cancer, one deletion affecting the entire BRCA1 gene was identified (0.8% of mutation-negative BRCA cases). For BRCA2 six deletions were detected (2.3%): del ex2, del ex10-12 and del ex15-16, this last one observed in four cases. Interestingly, deletions involving exons 15 and 16 seem to be frequent in our series accounting for 1.5% of all the rearrangements. It is important to note that four of these six cases with BRCA2 deletions were from families with co occurrence of female breast and male breast cancer.*

**P06.110****Ten years of BRCA1 and BRCA2 molecular diagnosis in Switzerland**

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Since 1999, our ISO certified laboratory is the Swiss reference laboratory for BRCA1 and BRCA2 genes analysis. We received samples from physicians involved in the Swiss (SAKK) network for cancer predisposition testing and counselling. Here, we describe for the first time 10 years of BRCA1 and BRCA2 genes molecular diagnosis in 1130 individuals from 900 distinct families from Switzerland with a personal and family history suggestive of genetic predisposition to breast/ovarian cancer.

Patients from high-risk families recruited from oncogenetics consultations in Switzerland (especially Geneva, Lausanne, Neuchâtel, Sion, Zürich, Basel, Bern, Lugano, St Gallen, Aarau) were screened for mutations in the entire coding regions of BRCA1 and BRCA2 by PCR-DHPLC or recently by PCR-HRM analyses. Abnormal profiles were characterized by DNA sequencing. Large BRCA1 and BRCA2 rearrangements were analysed by MLPA.

In 10 years, patients from 900 different families were screened for BRCA1 and BRCA2 genes. We found 175 mutation carriers (109 BRCA1; 66 BRCA2). Only 6 of these mutations were large rearrangements (4 BRCA1; 2 BRCA2). In the same time, 112 unknown variants (41 BRCA1; 71 BRCA2), and a large number of BRCA1 and BRCA2 polymorphisms were also identified. Some of these mutations, unknown variants and polymorphisms were not previously reported. On 230 relatives screened for the mutation found in the family, 106 were carriers.

The frequency of BRCA1 and BRCA2 mutations (near 20%) in breast and/or ovarian cancer families studied here is in the range observed in Caucasian families. No mutation seems to prevail in the Swiss population.

**P06.111****Mutation screening of BRCA1 exons 2, 11 and 20 in Bulgarian breast cancer patients**

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*Background: Studies on different populations worldwide demonstrate that germ line mutations in BRCA1 and BRCA2 cancer susceptible genes account for the majority of hereditary breast and ovarian cancers.*

**Materials and methods:** We have screened index cases from 65 high-risk breast cancer families for germ-line mutations in exons 2, 11 and 20 of the BRCA1 gene. Mutation analysis was performed by direct sequencing using 14 primer pairs covering the coding sequences and their intron-exon junctions.

**Results:** The founder mutations described in Ashkenazi Jews: 5382insC in BRCA1 exon 20 was observed in 6 patients from families with multiple cases of breast/ovarian cancer. Three of the patients have developed both breast and ovarian cancer, two were with early onset, and one had both early onset and bilateral breast cancer. In addition we found one unknown missense alteration in exon 11, codon 1037 (T>C), that leads to replacement of Val with Ala in patient with bilateral breast cancer developed by the age of 45.

**Conclusions:** It has been known that Ashkenazi Jews 5382insC founder mutation is spread all over Central and Eastern Europe and it is the most common in breast/ovarian cancer families from Hungary, Poland, Yugoslavia, Latvia, Italy, Spain, Greece and Russia. Our results suggest that the 5382insC might be also one of the most frequent mutations among breast/ovarian cancer families in Bulgaria.

One unknown missense mutation in codon 1037 (T>C) of BRCA1 exon 11 was also identified. The possible functional relevance of the mutation will be further studied.

#### P06.112

##### Descriptive study of French large rearrangements in *BRCA1* gene involving the promoting area

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The *BRCA1* gene is implied in the breast and ovarian cancer predisposition. 10-15% of deleterious mutations are large rearrangements involving one or several exons. In the French *BRCA1* database, 28 out of 403 deleterious mutations are large rearrangements. The 5' flanking region of the gene contains a *BRCA1* pseudo-gene which could ease rearrangements. Up to now, only three large rearrangements involving the promoting area of the gene have been described in the literature. In this study, we characterize 18 large rearrangements in this promoting region from French families with a zoom-in dedicated array-CGH (3107 oligonucleotides) and sequence the breaking points.

We found 11 never described events from 6kb to 238kb. There were 2 deletions from 5' to promoting region, 13 deletions from 5' to exon 2 and 3 deletions from 5' to other exons (exon 3, 17, full *BRCA1*). Two families have a rearrangement described in the literature (14kb and 37kb). A deletion to exon 2 was recurrent in 3 families. Two involved only the promoting area of the gene and not the coding sequence (5kb and 209kb).

The analysis of the breaking points revealed some redundant break regions which could improve the understanding of those events. Thanks to the efficacy of the dedicated array-CGH, the analysis confirms the diversity and the recurrence of the large rearrangements involving the promoting area.

#### P06.113

##### The *BRCA1/2* mutations and SNPs in ovarian cancer risk

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Germline mutations in the *BRCA1* and *BRCA2* genes confer increased susceptibility to ovarian cancer. We analyzed *BRCA1/2* mutations among ovarian cancer patients with familial history of breast/ovarian cancer and among unselected on familial history ovarian cancer patients. Among 101 unselected on familial history ovarian cancer patients in Russian population there were 17 cases with *BRCA1/2* mutations (16,8%). In order to find variants that may have influence on ovarian cancer risk three samples of patients on the base of mu-

tation analyses were formed and analyzed: ovarian cancer without *BRCA1/2* mutations, *BRCA1*-associated ovarian cancer and control sample. Several moderate penetrance cancer risk *BRCA1/2* variants were investigated. It was shown that genotype 203A/A in *BRCA2* gene was associated with increased ovarian cancer risk for both sporadic and *BRCA1*-associated ovarian cancer (OR=5,8; p=0,003). There was no confirmation of cancer risk modification by genotype 203A/A in a samples of patients with sporadic and *BRCA1*-associated breast cancer. The results demonstrate that defined genotype on SNPs may have influence on increasing ovarian cancer risk both sporadic and *BRCA1*-associated.

#### P06.114

##### The effect of *CHEK2* missense variant I157T on the risk of breast cancer in carriers of other *CHEK2* or *BRCA1* mutations

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**Purpose:** It is of interest to estimate the breast cancer risks associated with carrying two mutations because this information may be informative for genetic counselors and may provide clues to the carcinogenic process.

**Experimental Design:** We genotyped 7,782 Polish breast cancer patients and 6,233 controls for seven founder mutations in *BRCA1* and *CHEK2*.

**Results:** Of the 7,782 women with breast cancer, 1091 had one mutation (14.0%) and 37 had two mutations (0.5%). Compared to controls, the odds ratio for a *BRCA1* mutation in isolation was 13.1 (95% CI 8.2 to 21). The odds ratio was smaller for *BRCA1* mutation carriers who also carried a *CHEK2* mutation (OR = 6.6; 95% CI 1.5 to 29), but the difference was not statistically significant. In contrast, the odds ratio for women who carried two *CHEK2* mutations (OR = 3.9; 95% CI 1.5 to 10) was greater than that for women who carried one *CHEK2* mutation (OR = 1.9; 95% CI 1.6 to 2.1). The odds ratio for women who carried both a truncating mutation and the missense mutation in *CHEK2* was 7.0 (95% CI 0.9 to 56) and was greater than for women who carried the truncating mutation alone (OR = 3.3; 95% CI 2.4 to 4.3) or the missense mutation alone (OR = 1.6; 95% CI 1.4-1.9).

**Conclusion:** Our study suggests that the risk of breast cancer in carriers of a deleterious *CHEK2* mutation is increased if the second allele is the I157T missense variant.

#### P06.115

##### Preliminary genetic investigation of high-risk breast cancer patients in Armenia

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Mutations in high-penetrance genes *BRCA1* and *BRCA2* are associated with greater risk for developing breast cancer (BC). The benefit from prevention is not only increased by early detection of *BRCA* mutations in cases with strong family history, but also in early onset cases who make up a large fraction of all BC cases. This is the first report on preliminary results of high-risk BC cases in Armenia where the incidence of the disease is the highest in the South Caucasian region. In 2008, BC cases with family history or with early onset of the disease (before age 40 y.o.) consisted 6.3% and 25%, respectively, of all BC cases, with an increasing incidence of the latter during last two decades. In our pilot study we recruited 46 high risk BC cases (18 familial cases, 3 breast and ovarian cancer cases, 5 bilateral cases, and 20 early onset cases).

Using SSCP screening method, we have found 2 *BRCA1* missense substitutions in 2 patients (Q356R, S694L) and one synonymous *BRCA1* polymorphism in other 2 patients (L771L). This is the first report of S694V variant previously not reported in other populations in contrast to Q356R and L771L. It is notable that all carriers of the *BRCA1* changes were early onset cases with no family history of BC. Despite the lack of high sensitivity of the screening assay, the results indicate a proportion of early onset BC cases with *BRCA* mutations and the need to include them in the genetic counseling and mutations screening procedure.

**P06.116****Uptake of BRCA1/2 predictive testing and gender**

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Data concerning male uptake of BRCA1/2 predictive testing and pre-test characteristics in comparison to females are scarce. We investigated the cohorts of male and female applicants attending the Center of Human Genetics in Leuven during a 10-years period (1998-2007). Considering all unaffected siblings in the family of origin of the predictive test applicants, uptake of predictive testing for BRCA1/2 was 32% in males and 82% in females ( $p < .001$ ).

Males were significantly older than females. There was a trend ( $p < .10$ ) that more males than females had adult daughters. As expected, breast cancer related distress (IES) was significantly lower in men. Both males and females were a self-selected group with psychologically stronger individuals than average (SCL-90, UCL). Men were unanimously motivated (personal relevance of 12 motives rated on a Likert scale) by concerns for their daughters, significantly more than women. One third of them (versus 12% women) referred to child-bearing decisions.

Sixty percent of the adult daughters of mutation carriers (identified by a predictive test) had predictive testing versus 15% of adult sons. In the group of descendants 'having been tested or not' is significantly predicted by their own gender and age, but not by the gender of the parent with the mutation.

It is very likely that the lower uptake of predictive testing in males is due to their lower perceived personal risk.

**P06.117****CSCE screening for BRCA1 and BRCA2 mutations using the BioNumerics® software.**

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Conformation sensitive capillary electrophoresis (CSCE) is a sensitive method for mutation scanning (e.g. BRCA1/2 mutation detection). The method is more rapid and cheaper than full gene sequencing and uses electrophoretic mobility differences between homoduplex and heteroduplex DNA. By the use of multi-capillary sequencers, high throughput routine diagnostics becomes feasible, but requires the availability of reliable high throughput mutation detection software.

The BioNumerics® software enables automatic batch import of ABI. FSA files for high throughput processing and provides the necessary database environment as well as an adapted analysis tool for automatic mutation detection. Single peaks are identified as targets using a pre-set area, allowing advanced, cost saving, multiplexed settings. Peaks of the sample trace are compared to 'wild type' (WT) control traces using 5 parameters that examine primary peak shape (SRMS, MAXDIFF, DFH3 and DFH4) and look for secondary peaks (SECPK). Per PCR product, polymorphic variants can be defined. WTs can automatically be traced by the software, assuring easy accommodation of the software to any lab specific protocol or target. Results of automatic peak matching are displayed in overview reports with color indication for reference peaks, positive matches, mismatches, failed peaks and problem cases. Interactive click and zoom functions allow easy on-screen evaluation of (mis)matches.

Results are stored in the database. The system can learn from stored analyses using build-in tools that scan the database for limits of each target. Optimal average parameters can be calculated or set manually. Recently the protocol was validated by Eurogentest (Mattocks et al., in preparation).

**P06.118****The BRCA1/2 and TP53 mutations among patients with bilateral breast cancer**

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In previous work we found that familial breast cancer (BC) with cases of bilateral breast cancer (BiBC) may have some genetic peculiarities in comparison with BC families without BiBC. Now BiBC as familial so sporadic was investigated on BRCA1/2 and TP53 mutations.

There were 31 cases of BiBC due to BRCA1/2 mutations among 83 patients (37%). The BRCA1 gene mutations were predominant (27 of 31, 87%) with prevailing 5382insC (19 of 27, 70%). The percent of cases with BRCA1/2 mutations among familial BiBC (53%) was higher than among sporadic BiBC (12.5%). The young patients with the first BC onset up to 31 year without BRCA1/2 mutations were investigated on TP53 mutations. There were 2 cases with TP53 mutations (50%). For comparison, among 12 BC cases unselected on BiBC with the same age of first cancer onset there was 1 TP53 mutation (8%). It is not excluded that young age of BiBC onset may be marker of genetic connection with inherited TP53 mutations. In part supported by grant RFBR N 07-04-01602.

**P06.119****A novel BRCA2 mutation detected in a Greek family with an extended family history of breast cancer**

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Germline mutations in the BRCA1 and BRCA2 genes account for a large proportion of hereditary breast and ovarian cancers. In our laboratory, we routinely investigate BRCA1 and BRCA2 mutations by direct sequencing of the full coding region and partial intronic regions of the genes. In this report, we present a germline mutation in exon 14 of the BRCA2 gene which has not been previously reported at the BIC. This mutation was found in a healthy 34-year old woman with a strong family history of breast cancer where all females were diagnosed with breast cancer: her mother died from breast cancer at the age of 55 (date of onset 52), three maternal aunts were diagnosed with breast cancer before menopause, her maternal grandmother and two maternal granddaughters were also affected (date of onset after 60y). The same mutation was found in her only one affected living maternal aunt (date of onset 43). Interestingly enough, this patient had a son who died from leukemia at the age of 7y. The mutation is a two base pairs deletion, BRCA2 c.7032\_7033delCA GenBank NM\_000059.3, (p.Asn2346SerfsX13) leading to a change in the translational reading frame of the gene (frameshift mutation) and the appearance of a premature stop codon, linked to the expression of a truncated protein. Considering the age of onset of the disease in most affected women in this family and the number of the affected members we believe that this deletion plays an important role in the development of breast cancer in this family.

**P06.120****Two distinct origins of 3036delACAA BRCA2 mutation in Castilla-León (Spain).**

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The distribution of BRCA2 germline mutations in breast/ovarian cancer families varies among different populations. Founder mutations have been used to explain the high frequencies of disease-associated mutations in specific human populations, but for characterize a recurrent mutation as founder it is necessary to demonstrate that it occurred only once in history. The 3036delACAA mutation has been found worldwide (BIC database). This mutation has been identified in almost 45 non-related Spanish families, 25 of whom had ancestors in Castilla-León. Although this mutation is placed in a potential hot spot mutations region we hypothesized a unique origin in our families.

Twenty-one 3036delACAA positive were genotyped at eight STRs and two SNPs covering 1.34 Mb around the BRCA2 gene. The equation  $G = \log \delta / \log(1-\theta)$  was used for estimate the mutation age in generations. The software Quikfold was used to predict secondary structures in which the mutation is included.

Two conserved haplotypes were observed in ten (eastern provinces) and nine (western provinces) families, whereas other two families share another different haplotype. The estimated mutation age depends on the haplotype used, resulting in about 95 generations (ten families) or 187 generations (nine families). *In silico* analysis shows a complex secondary structure where the mutation is located into a hairpin loop in a single-stranded segment exposed to spontaneous mutagenesis.

The 3036delACAA mutation seems to have multiple occurrences in

the past and it is probably positioned in a hot spot point for mutations. The two different haplotypes reflects diverse historical origins in the east and west Castilla-León region.

#### P06.121

##### **Detection and characterization of new large deletion of exon 3 in the *BRCA2* gene among a French breast cancer family.**

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Germ-line mutations in two genes, *BRCA1* and *BRCA2* genes, are the major contributors to hereditary breast/ovarian cancer. Nowadays, large rearrangements have been described regularly in *BRCA1* with approximately 10-15% of the mutations in this gene. In contrast, large genomic rearrangements in the *BRCA2* gene have been rarely reported.

During the comprehensive screening of breast/ovarian cancer families for germ-line mutation in these genes, we detected a deletion of exon 3 in *BRCA2* in a breast cancer family, using the QMPSF (quantitative multiplex PCR of short fluorescent fragments) and confirmed by MLPA (multiplex ligation-dependent probe amplification) methods for detection of large genomic rearrangements. This mutation was characterized by high-resolution oligonucleotide array-CGH technology which estimated the size of the deleted region and helped to find the breakpoint. Precise determination of the size and identification of the breakpoint were obtained with a specific PCR and the deleted sequence was different from previous reports. Moreover, analysis of transcripts revealed the only skipping of exon 3. Since this deletion is in-frame, the deleterious impact is largely discussed in the literature. Despite a limited number of cases in this family, the segregation data seemed to be consistent with a causal effect of the mutation.

In addition to conventional DNA diagnostic testing by sequencing, familial breast cancer patients can benefit from searching for large rearrangements in the *BRCA2* gene. The in-frame deletion of exon 3 needs additional studies to investigate its contribution to hereditary breast/ovarian cancer.

#### P06.122

##### ***BRCA1/2* screening results: Review of molecular data concerning Portuguese high-risk breast/ovarian cancer families**

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**Introduction:** Although *BRCA1* and *BRCA2* are the genes most frequently involved in familial aggregation of breast/ovarian cancer, mutations in other genes like *TP53*, *PTEN*, *ATM*, *CHEK2* and *PALB2* could also cause breast cancer risks. Taken together, the known susceptibility genes account for less than one third of breast cancer families undergoing genetic testing while other gene defects remain to be discovered [EMQN guidelines 2007].

**Patients and methods:** Review of all patients for whom *BRCA1/2* screening is complete. All patients underwent pre and post-test counselling and are pre-screened for the *BRCA2* Portuguese founder mutation [Machado et al, 2007]. Negative patients are further analysed by CSCE and samples with a different pattern are sequenced. Previous to the CSCE optimization, DNA samples were analysed by CSGE.

**Results:** Two hundred and forty nine families were fully screened for *BRCA1/2* mutations and 61 positive patients were detected (10 *BRCA1* and 51 *BRCA2* mutations, including 28 families with the founder mutation), which corresponds to a 25% detection rate. The range of mutations and sequence variants as well as their frequency in this set of patients were analysed, allowing for a better characterization of the breast/ovarian cancer high risk families from the Central and Southern regions of Portugal. Depending on pedigree reanalysis *BRCA1/2* negative families are being screened for other gene mutations (*p53* and *PTEN*).

**Conclusion:** From 249 Portuguese high-risk families, 61 presented deleterious *BRCA* mutations. As expected, several neutral or unknown variants were also detected, posing a significant challenge for counselling.

#### P06.123

##### **Identification and characterization of large genomic rearrangements in *BRCA1*, *BRCA2* and *CHEK2* genes**

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Large genomic rearrangements are estimated to account for about 5-10% of all disease-causing mutations in *BRCA1* and *BRCA2* genes in patients with hereditary breast and ovarian cancer syndrome (HBOC). To screen for such rearrangements in patients with HBOC, and as a first step in our genetic testing workflow, we use MRC-Holland Multiplex Ligation-dependent Probe Amplification (MLPA). We have used this technique in a set of 310 independent patients and we have detected 9 different copy number alterations corresponding to 3% of the studied samples and about 15% of all identified mutations in *BRCA1* and *BRCA2* genes in our cohort. As commercial MLPA tests are not suitable to determine the specific breakpoints or to define the exact extension of the rearrangement, we have applied a set of different complementary techniques in order to better characterize these genetic alterations. We have used long-range PCR amplification, RNA analysis, SNP array chips, not commercial MLPA probes and FISH analysis to fully define the extent and mechanism of each of the identified alterations. Briefly, in *BRCA1* we have characterized 6 rearrangements: deletion of E22, deletion of E9-E24, deletion of E16-E23, deletion of E1-E13, deletion of E1-E2, duplication of E1-E2. In *BRCA2* we studied a deletion of E15-E16 and a deletion of E1-E24 and in *CHEK2* we have identified a complete gene deletion. In addition, it is worth to mention that MLPA demonstrated to be useful to identify point mutations located within probe sequences.

#### P06.124

##### **Cost-effectiveness analysis of prophylaxis programme in women with a family history of breast cancer and *CHEK2\*1100delC* heterozygosity in the Polish health-care system**

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**Aim of the study:** economic evaluation of prophylaxis programme starting at 25 years old women with a family history of breast cancer and *CHEK2\*1100delC* heterozygosity versus no prophylaxis.

**Methods:** Cost-effectiveness analysis was performed using modelling technique. Two cohorts were studied: without prophylaxis, and with prophylaxis. Data on life expectancy, breast cancer risk, efficacy of prophylaxis and medical costs were obtained from published literatures. The cohort simulation started with 25-year-old women and projected direct medical costs and outcomes over patients lifetimes. Effectiveness was measured as life years gained (LYG). Only direct medical costs (breast cancer prophylaxis and treatment of breast cancer I-IV stages) were included, assessed from health-care payer perspective and reported in PLN (1 EUR= 4.5 PLN in 2009). 5% and 3.5% discount rate was used for cost and effectiveness, respectively. Sensitivity analyses to treatment patterns, efficacy of prophylaxis and costs were performed.

**Results:** The total lifetime costs/patient were estimated to be 4453 PLN (discounted: 1318 PLN) in no prophylaxis arm and 5380 PLN (discounted: 2135 PLN) in prophylaxis arm. The life expectancy generated with prophylaxis was 75.43 vs. 70.5 for no prophylaxis (without discounting) and 48.01 vs. 46.79 (discounted), respectively. This results in ICER for prophylaxis of 187.7 PLN/LYG (without discounting) and 672.7 PLN/LY (discounted). Results were robust to sensitivity analyses.

**Conclusion:** Breast cancer prophylaxis programme for women with a family history of breast cancer and *CHEK2\*1100delC* heterozygosity compared to no prophylaxis improves survival and is highly cost-effective in the Polish health-care system.

## P06.125

**Identification of a recurrent BRCA1 mutation in a north-eastern Romanian population**

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Introduction: We started the first characterization of hereditary breast and ovarian cancer risk in north-eastern Romania, searching for mutations in the cancer predisposition genes BRCA1 and BRCA2 in high-risk families.

Patients and methods: We identified and recruited 15 hereditary breast and ovarian cancer (HBOC) families, with at least 3 cases of epithelial breast or ovarian cancer within the same family line. All patients agreed by written informed consent. DNA was extracted from peripheral blood. The entire coding sequence of both genes was analysed using amplification and Sanger sequencing. BRCA1 was screened for large deletions and duplications by MLPA. Multiplex-PCR was used to screen for certain mutations recurring in different populations.

Results: We observed a recurring BRCA1 mutation in exon 20, c.5266dupC. This mutation was found in two different HBOC families, with different breast/ovarian cancer familial history and without any apparent degree of relatedness.

The C duplication at position 5266 from initiator ATG creates a stop codon at position 5484, truncating the BRCA1 protein upstream of the C-terminal BRCT domain. This domain is responsible for molecular interactions with BRCA2 and p53, and is involved in transcriptional activation. Its deletion can be considered responsible for functional loss of the BRCA1 tumour suppressor in cancer cases.

Conclusions: This outcome, the first one in Romania, could open the way for a population study to determine the frequency of c.5266dupC in the Romanian population, and for an eventual founder effect. This could also develop in Romania the oncogenetic approach and follow-up of BRCA mutations bearers.

## P06.126

**DNA-methylation changes in metastatic breast cancer**

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Aberrant DNA methylation is an early event during neoplastic transformation. Thus DNA methylation changes might be potent tumormarkers for diagnosis and specific methylation patterns might also be used for classification and prediction of disease outcome as well as for the development of metastases.

To investigate methylation changes on a genome-wide scale we established a specific amplification protocol based on methylation-sensitive restriction digestion. This amplification does enrich the methylated DNA and enables whole genome methylation screenings.

We used this protocol for analysing the DNA methylation status from primary tumors of breast cancer patients with vs. without metastases (10y follow up) on Agilent 244k human CpG island microarrays covering 27 800 CpG-islands.

From those chip-experiments we defined a group of potential marker genes exhibiting significantly different methylation patterns between both patient groups and performed KEGG pathway analyses. There we found enriched numbers of these genes belonging to cellular pathways associated with angiogenesis and metastasis.

## P06.127

**The survey of gene mutations in P53 gene in patients of malignant breast cancer by PCR-SSCP method.**

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Background: Mutations in the p53 tumor suppressor gene are the most common genetic alterations in human malignancies. These mutations founded 40-50% in breast carcinomas. In the present study, we analyzed P53 gene mutations (exon 5-9) by PCR-SSCP method in 56 women patients of breast cancer.

Materials and methods: DNA extraction from samples (formalin-fixed

and paraffin-embedded or fresh tissue samples) of breast cancer patients were done by standard Phenol chloroform method and stored in -20°C. For mutation detection, exons 5-9 amplified by polymerase chain reaction (PCR) and then specific electrophoresis (single-strand conformational polymorphism SSCP).

Results: Abnormal movement of PCR products band in SSCP gel that stained with silver nitrate reported as mutation. We found three mutations (one in exon 5, one in exon 8 and one in exon 9) in patients.

Discussion: p53 Mutational analysis by PCR/SSCP method deserves to be critically studied as a diagnostic criterion in patients with indeterminate or suspicious cytology. Validation studies should be performed to test p53 mutations, as molecular diagnostic markers in breast cytology specimens. Detection of P53 gene mutations can be helpful in pre diagnosis and prevention of breast cancer and so in treatment. These mutations occur in normal or benign breast tissue but resolutions of this role in the pathogenesis of breast cancer will require long-term follow-up studies.

## P06.128

**Evaluation of epigenetic changes in TIMP3, GSTP1, BRCA1, E Cadherin and P16 genes in breast cancer tumors and it's relation with grade of tumors**

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Epigenetic changes in breast cancer often occur and mostly happen in promoter's CpG islands that affects the genes expression state (mostly tumor suppressor genes), in this study we performed the evaluation of these alterations in GSTP1, P16, BRCA1, E-cadherin and TIMP3 genes for methylation pattern and it's relation with kind and grade of tumor. Total 100 sample: 50 breast cancer patient with different kind of tumors selected and 50 control tissue was obtained from same patient and breast (adjacent area of tumor tissue) after pathological diagnosis, and we performed bisulfite sequencing, immunohistochemistry and western blotting methods for evaluation of promoter methylation pattern, gene presence and expression status respectively. Data analyses will perform with cox-regretion statistical methods with ( $p < 0.005$ ) criteria for significant variations between tumor and normal tissue in SPSS.13 version software. Results will show the significant difference between methylation pattern in promoter regions and expression state between normal and tumorous tissues and relation with grade and kind of tumor in epigenetic changes with above mentioned genes.

## P06.129

**Polymorphisms in ERCC2 K751 was Associated with Breast Cancer Risk**

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Numerous studies are addressing associations of polymorphisms in DNA repair genes and cancer risks [1] because accurate and efficient DNA repair is crucial to genomic integrity and fidelity.

ERCC2 are important in DNA nucleotide excision repair and lie on chromosome 19q13.3. We genotyped constitutive variants ERCC2 K751Q and R156R in approximately 400 adults with breast adenocarcinoma and 160 controls of Iranian women.

Totally 560 Iranian sporadic breast cancer affected women compare to control group were studied by PCR-RFLP for ERCC2 variant.

Our results showed that heterozygote genotype ERCC2 (751) has the highest frequency in both groups (21.8 in patients and 8.7 in control group).

The Genotype ERCC2 (751) GT were most risk factor in our population. [GG / TT odds ratio, 5.90 (95% confidence interval; CI, 11.45-12.15)  $p=0.001$ , GG / GT odds ratio, 4.737 (95% CI, 9.03-9.92)  $p=0.029$ ], TT / GT odds ratio, 5.465 (95% CI, 10.41-11.45)  $p=0.002$ ].

We conclude that not only G/G and T/T in our patients was not associated with breast cancer risk but also there is a relation between presence of G/T and increasing of breast cancer risk.

**P06.130****Spectrum and incidence of BRCA1 and BRCA2 mutations in the Republic of Ireland - An Audit**

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Comprehensive mutation screening of BRCA1 and BRCA2 has been available to Irish breast cancer families since 2005 via our Centre. We present an audit of the data generated following bi-directional sequencing and MLPA of BRCA1 and BRCA2 in 462 breast/ovarian cancer patients to date. In total, pathogenic mutations have been identified in 154 families (33%). The spectrum of these mutations comprises non-sense (BRCA1: 21, BRCA2: 3), frameshift (BRCA1: 30, BRCA2: 56), splice-site (BRCA1: 7, BRCA2: 2), substitution (BRCA1: 5, BRCA2: 6) and large deletions (BRCA1: 22, BRCA2: 2). Overall, the incidence of large deletions was found to be approximately 5% in the patient group screened to date, accounting for approximately 15% of the total mutation incidence. Variants of unknown significance were identified in 28 families and of these, 6 were present with a pathogenic mutation. Eight mutations have been identified in more than 3 apparently unrelated families: BRCA1: p.E143X (19), c.1294\_1333del40 (7), exon 3 deletion (4), exon 21-24 deletion (4); BRCA2: c.8525delC (9), c.983del4 (6), c.2117delC (7). In addition, a large deletion encompassing exons 1-23 of BRCA1 has been identified in 4 families. Haplotype analysis for a possible founder effect is underway for some of these recurrent mutations.

Results to date indicate that a significant proportion of hereditary breast/ovarian cancer in Ireland are attributable to mutations in BRCA1 and BRCA2 and that large deletions in BRCA1 occur in approximately 5% of Irish breast cancer families, an incidence that is in line with that observed in other populations (2-10%).

**P06.131****Prevention or Surveillance - a study among BRCA1/2 mutation carriers**

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This study aimed to investigate the socio-demographic and clinical characteristics of BRCA1/2 carriers opting for preventive surgeries. Of 148 BRCA1/2 carriers, 111 (75%) had unilateral breast cancer (BC) and 37 (25%) were asymptomatic women. The study protocol integrated socio-demographic and clinical follow-up; and psychological questionnaires. Prophylactic oophorectomy was reported by 84 (75.7%) and 25 (67.6%) unilateral BC patients and asymptomatic women, respectively. Comparable mean ages at oophorectomy (47±9 years) were noted for both BC patients and asymptomatic women. However, different mean ages of 44±10 and 33±9 years recorded for BC and asymptomatic surveillance groups, respectively ( $p<.05$ ). High state of anxiety characterized the surveillance group (BC patients-37.6±22.4 and asymptomatic-43±7.3) compared to the preventive oophorectomy group (BC patients and asymptomatic carriers 32±8.7). Among BC patients, 47 completed the study protocol. Of these, seven (15%) underwent contralateral prophylactic mastectomy and 12 (25%) performed bilateral mastectomy, however, following malignant findings. None of the asymptomatic carriers chose preventive mastectomy. Comparable state of anxiety characterized all BC groups. Higher BC risk perception was reported by patients who underwent conservative breast surgery (37%) compared to patients opting for bilateral or contralateral preventive mastectomy (20% and 24%, respectively). Prophylactic oophorectomy reduced the perceived risk for ovarian cancer to below 5%, however, contralateral preventive mastectomy reduced the perceived risk for BC to 20%. Prophylactic surgeries reduced the risk perception for breast and ovarian cancer, the highest levels of anxiety being predominantly expressed by BC and asymptomatic surveillance groups.

This study was supported by the Israel Cancer Association, grant 20070091-c.

**P06.132****A one step BRCA1 and BRCA2 point mutation and large rearrangement detection using High-Resolution Melting curve analysis associated to qPCR analysis (qPCR-HRM)**

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High resolution melting (HRM) of DNA is a simple method for mutation scanning which monitors the fluorescence of double strand DNA with saturating dye. Performing HRM on real time thermocycler, enables semi-quantitative analysis (qPCR) to be associated to HRM analysis for detection of both large gene rearrangements and point mutations (qPCR-HRM). We evaluated this method of mutation screening for the two major breast and ovarian cancer susceptibility genes BRCA1 and BRCA2. Screening of these two large genes are time-consuming and must include exploration of genomic rearrangements that represent 5 to 15% of the alterations observed in these genes.

We evaluated the sensitivity of the HRM technology by analysing 201 known variants scattered over all amplicons of BRCA1 and BRCA2 genes and the sensitivity of quantitative PCR by analysing seven large gene rearrangements involving exons of BRCA1 or BRCA2; 100% of the variants were detected including rearrangements. Furthermore, a retrospective study was done with 44 patients previously BRCA1 tested by DHPLC, all the variants were detected by HRM analysis. Finally, a prospective study was done with 165 patients allowing 22 deleterious mutations, 16 unclassified variants and two large rearrangements to be detected. qPCR-HRM is a simple, sensitive and rapid method that does not require modified PCR primers. Thus, this method allows in one step the detection of point mutation and gene rearrangements.

**P06.133****Comparative analysis of BRCA1 gene expression in ovarian cancer tissue and in tumor environment.**

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It is known that BRCA1 gene expression is low in cells of breast cancer in comparison with normal tissue. These results are considered as indication of BRCA1 gene expression significance in breast cancer development. However data on BRCA1 expression in ovarian cancer tissue not enough.

We investigated BRCA1 gene expression in samples of ovarian cancer tissues and also in tissues of tumor environment by RT-PCR. Comparative analysis of BRCA1 gene expression in the tumor of this localization and in tissues of tumor environment was conducted for the first time. The BRCA1 expression level was determined comparative to expression of GAPDH gene. Each measurement was repeated 3-5 times. For the first time it was shown that in 6 of 10 ovarian cancer samples BRCA1 gene expression was decreased in tissues of tumor environment in comparison with tumor tissue. This result is important in the light of data about decreased BRCA1 gene expression significance and also about microenvironment influence on the processes of malignant tumor onset and development. The detection of BRCA1 gene expression in the tissue sample with distant from tumor location is in process now and will be reported. In part supported by grant RFBR N 07-04-01602.

**P06.134****Estrogen receptor- $\alpha$  gene, codon 594 (G3242A) polymorphism among Iranian women with breast cancer**

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Evidence suggests that *ESR1* gene polymorphism has been found to be associated with breast cancer and clinical features of the disease in Caucasians. A case-control study was conducted to establish a database of *ESR1* polymorphisms in Iranian population in order to compare Western and Iranian distributions and to evaluate *ESR1* polymorphism as an indicator of clinical outcome. The *ESR1* gene was scanned in Iranian patients newly diagnosed invasive breast tumors,

(150 patients) and in healthy individuals (147 healthy control individuals). PCR single-strand conformation polymorphism technology and direct sequencing was performed.

The frequency of genotype 01 in codon 594 (ACG → ACA), (G3242A), exon 8 was significantly higher in breast cancer patients (48.0%) than in control individuals (1.4%;  $P=0.001$ ). The allele 1 in codon 594 was significantly more common in breast cancer patients with age at menarche  $<=12$  (40.8%) than in those which their menstruation began at older than 12 years old (23.9%;  $p=0.002$ ). The allele 1 in codon 594 exhibited, the greater the frequency, the lesser the likelihood of LN metastasis. Our results demonstrated that this particular SNP marker may increase accuracy in predicting LN. Therefore, this SNP marker further increased predictive accuracy in Iranian population.

These data suggest that *ESR1* polymorphisms are correlated with various aspects of breast cancer in Iranian *ESR1* genotype, as determined during pre-surgical evaluation, might represent a surrogate marker to increase predicting breast cancer in Iranian population.

#### P06.135

#### Genetic cancer risk modifiers in hereditary breast and/or ovarian cancer families

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Introduction: Variability observed in penetrance, age of onset, and site of the tumor, both among and within *BRCA* families suggests that, other, low-penetrance, genetic variants modify the cancer risk.

Objective: To study the effect of six polymorphisms, recently described as being risk modifiers of sporadic breast cancer (BC) or ovarian cancer (OC) in *BRCA* families.

Methodology: We recorded the cancer history (tumor site, age of diagnosis) of 548 women (293 carriers, 255 non-carriers) from 125 *BRCA* families (72 *BRCA1*, 53 *BRCA2*). The polymorphisms genotyped were: +331G/A and PROGINS, localized in the progesterone receptor gene, *CASP8* D302H, *CASP8* -652 6Nins/del, *FGFR2* (rs2981582) and *TNRC9* (rs3803662). Familial clustering was taken into account in the statistical analyses.

Results: Two polymorphisms modified the risk of OC in *BRCA1* and *BRCA2* families: +331 G/A heterozygous genotype increased the risk (OR:2.41, 95%CI: 0.98-5.96,  $p=0.056$ ), while *FGFR2* had a protective effect (OR:0.52, 95%CI: 0.28-0.96,  $p=0.037$ ). *FGFR2* was also significantly associated with increased risk of bilateral BC (OR:2.67, 95%CI: 1.45-4.92,  $p=0.002$ ), whereas *CASP8* -652 6Nins/del had a trend towards a protective effect, restricted to *BRCA1* mutation carriers (OR:0.71, 95%CI:0.48-1.05,  $p=0.084$ ). Furthermore, PROGINS was significantly associated with BC among the *BRCA2* non-carriers (phenocopies) (OR:8.17, 95%CI: 2.17-30.76,  $p=0.002$ ).

Conclusions: We have found evidence that certain polymorphisms involved in hormone mediated cell proliferation and apoptosis modify the BC and OC risks in *BRCA* families. This may be relevant for an individual assessment of the most suitable preventive option, among those currently available, for each of those patients.

#### P06.136

#### Detection of HER-2/neu Gene Amplification in Breast Carcinomas using Fluorescence in-situ hybridization Comparison with Immunohistochemical Results

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The aim of our study was to evaluate the value of Fluorescence in-situ hybridization (FISH) in the determination of her2/neu amplification status of human breast carcinomas by comparing immunohistochemistry (IHC) results from the same sample. A series of 20 ductal carcinomas were analyzed by FISH for HER2/neu gene and chromosome 17 copy number using Vysis Path Vysis Probe and by IHC using a polyclonal antibody A0485 to HER2 protein in corresponding formalin-fixed, paraffin-embedded tissues sections. HER2/neu protein overexpression of moderate (2+) or high (3+) intensity based on IHC was detected in 10 carcinomas and was 2+ in 2 carcinomas and 3+ in 8 carcinomas.

According to the HER2/CEP17 RATIO, 6 tumors were judged to have HER2/neu DNA amplification, with 2 having low-level of amplification ( $\geq 2.2$  but  $<3$  folds) and 4 having high-level of amplification ( $\geq 3$  folds). Our results showed significant concordance between FISH and IHC. THE HER2/CEP17 RATIO of  $\geq 2.2$  was concordant with IHC findings of 2+/3+ in 60% of carcinomas. High level of amplification was detected in 5 of 8 IHC3+ cases (62.5%), but in only 1 of 2 IHC 2+ cases (50%) and 0 of 10 IHC 1+/0 cases. All cases with high level of amplification showed an IHC score of 3+ but not all cases with overexpression of HER2 showed amplification by FISH. Based on these results, we consider FISH to be the gold standard for detecting of HER2/neu status in breast cancer and were applied in all cases.

#### P06.137

#### Increased susceptibility for breast cancer development with eRF3 - 12 GGC allele

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The involvement of translation factors in cancer development it is now widely recognized. Moreover, the components of the translation machinery that are deregulated in cancer cells may become targets for cancer therapy. The eukaryotic Release Factor 3 (eRF3) is a GTPase that associates with eRF1 in a complex that mediates translation termination. eRF3 first exon contains a (GGC)n expansion coding for proteins with different N-terminal extremities. In the present work, we show that the longer allele (12-GGC) is present in 5.1% (7/137) of the breast cancer patients analysed and is absent in the control population (0/135), corresponding to an 18 fold increased risk for cancer development, as revealed by Odds Ratio analysis. mRNA quantification suggests that patients with the 12-GGC allele overexpress eRF3 in tumour tissues relative to the normal adjacent tissues. However, using an *in vivo* assay for translation termination in HEK293 cells, we do not detect any difference in the activity of the eRF3a proteins encoded by the various eRF3 alleles. Although the connection between the presence of eRF3 12-GGC allele and tumorigenesis is still unknown, our data suggest that the presence of the 12-GGC allele provides a potential novel risk marker for various types of cancer.

#### P06.138

#### Molecular epidemiology of breast cancer in the Azores Islands: Preliminary results for the TSER polymorphism

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The Thymidylate synthase enhancer region (TSER) is an untranslatable region on the 5' end of the Thymidylate synthase gene (TYMS), located on 18p11.32. This region has a polymorphic tandem repeat of 28 bp, with 2 or 3 repeats as the most common alleles. Previous studies reported an association between this polymorphism and the amount of protein expressed which has an effect on the success of the 5-FU-based chemotherapy in cancer. The 5-FU is one of the drugs used on the breast cancer therapy. We report the preliminary results of a genotyping survey of 96 Azorean BC and 96 cancer free control individuals. DNA was extracted from peripheral blood lymphocytes and genotyped by standard PCR followed by electrophoresis. The allelic and genotypic frequencies as well as departure from Hardy-Weinberg equilibrium were obtained using Genepop software. The current data were compared with data available from other populations using the T-test implemented on SPSS. We identified the alleles TSER\*2, TSER\*3 and TSER\*4. Allele TSER\*4 was found in both cases and controls. The most common genotype was TSER\*3/TSER\*3, for cases and controls, but with a higher frequency in controls than in BC patients. Genotypic frequencies were not in conformity with Hardy-Weinberg expectations. The comparison between the healthy Azoreans with cancer free individuals from other populations showed that our population is more similar to Caucasians and Africans than to the Asians. This preliminary study will be used as stepping-stone for a wider pharmacogenetics study of the BC patients in the Azores.

## P06.139

**The role of estrogen receptor alpha 5' UTR methylation in pathogenesis of Iranian patients with breast cancer**

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**Introduction:** Methylation pattern in promoter region of many genes are proved to be modified in cancers. 5' UTR of estrogen receptor alpha has been shown to be differentially methylated in some patients with breast cancer and is believed to be a marker in early diagnosis, prognosis and treatment. On the other hand, known genetic causes of breast cancer like BRCA genes couldn't explain early breast cancer in Iranian patients with breast and/or ovarian cancers. Our objective was to explore the role of epigenetic modification in ER $\alpha$  5' flanking region in Iranian patients with breast cancer.

**Materials and methods:** Methylation Specific PCR on sodium bisulfate treated DNAs of peripheral blood and tissue samples from 35 patients with breast cancer were performed. Primers were selected to be specific for 2 known CpG islands in 5'UTR of ER $\alpha$  either for methylated or unmethylated status.

**Results:** We found that in 53.85% of primary breast cancers CpG island I is methylated and in 80.77% CpG island II is methylated. The methylation status of these CpG islands in blood was 100% and 87.88% respectively.

**Discussion:** We find a trend to methylation in breast tissue of Iranian patients with breast cancer. Our result show a methylation tendency in breast tumors compared to those reported in the literature. We found a high rate of methylation in peripheral blood which is in concordance to other studies. Our results are in concordance to the studies which showed a role of ER alpha methylation in pathogenesis of breast cancer.

## P06.140

**Association of COX-2 -765G→C promoter variants with colon cancer from Iran**

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Cyclooxygenases-2 enzyme (COX-2) converts arachidonic acid to prostaglandins. The COX2 gene plays an important role in inflammation and carcinogenesis process. Previous studies indicated that polymorphism of this gene is associated with inflammatory diseases and several types of cancer. It has been shown that the COX-2 -765G > C promoter polymorphism have lower promoter activity and decreased COX-2 expression. In addition, genetic polymorphisms of the COX-2 gene could alter the response to Nonsteroidal anti-inflammatory drugs (NSAIDs). The aim of this study is to determine the association of the COX2 gene polymorphism with development of colon cancer. Colorectal cancer is the third cause of the cancer-related death in the world. To understand the etiology of colon cancer in Iran, we initiated a study to investigate genetic polymorphism (-765G→C promoter variants) of Cyclooxygenase-2 (COX-2) among Iranian colon cancer patients, the COX-2 -765G > C promoter genotypes were determined by polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) analysis in 16 Iranian colon cancer patients and 193 healthy individuals. The frequency of C allele was demonstrated more among colon cancer patients. Range of C allele frequency was 22.5% in healthy individuals to 50% in patients. Significance difference in COX-2 allele distribution was observed between patients and healthy individuals (P value <0.01). Our findings suggest that COX-2 genotype may play an important role in colon carcinogenesis among Iranian patients. In addition, according to these results we suggest that the NSAIDs can be used for reduction of colon cancer incidence among Iranian populations.

## P06.141

**Immune modulators added to 5-fluorouracil therapy - impact on microsatellite unstable colon cancer**

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**Background:** A significant proportion of CRCs is characterized by mismatch repair defects, leading to a high mutation rate in repetitive DNA sequences (MSI). It has been shown that patients with MSI tumours had a better prognosis, but benefit from 5-fluorouracil-based chemotherapy was questionable. The aim of our study was to analyse the effect of immune modulatory substances in addition to 5-fluorouracil (5-FU) for treatment of stage III colon cancer.

**Material and Methods:** 594 patients were prospectively randomized into four arms of adjuvant 5-FU treatment: arm 1 - 5-FU alone; arm 2 - 5-FU plus levamisole; arm 3 - 5-FU plus interferon; arm 4 - 5-FU plus levamisole and interferon. For microsatellite analysis, we used 5-10 mono- or dinucleotide repeat markers which were PCR-amplified and separated on an ABI PRISM® 310 Genetic Analyzer. Frameshift mutations in coding repeats of BAX and TGF $\beta$ -RII were analyzed similarly. Overall and disease-free survival rates were calculated.

**Results and Conclusion:** MSI was detected in 7.6% of eligible tumours. Survival rates were not different between patients with stable or unstable tumours. Patients with MSI tumours and BAX mutation seemed to benefit from the addition of levamisole to 5-FU. In patients with MSI tumours the cumulatively delivered 5-FU dose per patient demonstrated to have a statistically significant impact on survival. Optimal treatment of patients with MSI tumours is still under discussion; additional markers may improve patient outcome and prevent unnecessary side effects. Prospective studies evaluating molecular markers are necessary to identify those patients who may benefit from chemotherapy.

## P06.142

**Association of GSTM1, GSTT1, GSTP1 and CYP2E1 single nucleotide polymorphisms with colorectal cancer in Iran**

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Colorectal cancer a major cause of morbidity and mortality both globally and in Iran. The aim of this study was to determine the association between genetic polymorphisms of cytochrome P4502E1 (CYP2E1), glutathione S-transferases P1, M1 and T1 (GSTP1, M1, T1) and susceptibility to colorectal cancer (CRC).

Genotyping of CYP2E1 and GSTP1, GSTM1, GSTT1 was performed by the use of pyrosequencing. One hundred cases and healthy controls were enrolled into this study.

Mean GSTT1 polymorphism type was significantly ( $P < 0.01$ ) higher in cases as compared to controls ( $P < 0.0001$ ; OR, 2.43; 95% CI, 1.47-4) on the other hand there is no significant association between GSTM1, CYP2E1, GSTP1 and colorectal cancer.

GSTs measurement may be useful as a colorectal marker in colorectal cancer and biopsies obtained at colonoscopy can be used to measure tumor markers.

Gene	Polymorphism	Percent					
		Normal		Heterozygote		Homozygote	
Control	Cases	Control	Cases	Control	Cases	Control	Cases
<b>CYP2E1</b>	AG Exon4	100	95.7	0	4.3	0	0
	AG Promoter Upstream	100	97.1	0	2.9	0	0
<b>GSTM1</b>	AT Exon8	87	89.9	13	8.7	0	1.4
	CG Exon8	94.2	97.1	5.8	2.9	0	0
<b>GSTP1</b>	CT Exon8	81.2	88.4	18.8	11.6	0	0
	GA Exon5	47.8	52.2	46.4	40.6	5.8	7.2
<b>GSTT1</b>	GC Exon5	62.3	58	31.9	36.2	5.8	5.8
	TC Exon6	54.1	69.6	15.9	29	0	1.4
<b>GSTM1</b>	AG Exon4	65.7	95.7	4.3	4.2	0	0
	AG Exon4	55.1	39.1	34.8	24.6	10.1	36.2
<b>GSTP1</b>	CT 3' UTR	100	100	0	0	0	0
	AG Exon4	85.5	75.4	10.1	18.8	4.3	5.8

#### P06.143

#### Role of polymorphisms of DNA repair genes and risk of colorectal cancer.

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Despite great progress in the knowledge of the biology of malignancies, the genetic background of most cases of sporadic colorectal cancer (CRC) cases remains unclear. We focused on polymorphisms in DNA repair genes as the modulating factors in CRC-risk.

We considered genes involved in DNA-repair pathways: BER (OGG1 Ser326Cys, XRCC1 Trp194Arg and Arg399Gln); NER (XPA (-4)G/A, XPC PAT, (11) C/A and Lys939Gln, XPD Ile199Met, Asp312Asn and Lys751Gln, XPF Arg415Gln, XPG Asp1104His, ERCC1 C118T); HR (NBS1 Glu185Gln, Rad51 135G/C, XRCC3 Thr241Met).

The study group consisted of 133 patients diagnosed with sporadic CRC, while the control group of 100 elderly non-cancer volunteers. Genotyping was performed by PCR and PCR-RFLP. Fisher's exact test with a Bonferroni correction for multiple testing were used to assess differences in genotype distribution, linkage analysis and statistical significance of the correlations.

Our study revealed an association between the CRC-risk and polymorphisms in genes involved in NER pathway, with XPC (C/A) polymorphism as a main alteration. Moreover, our study showed an association among CRCs characterized by microsatellite instability and polymorphisms in DNA-repair genes (NER and HR pathways).

Our results indicate that however, investigated polymorphisms are not a major factor modulating an individual risk of CRC development, a network of genetic alterations may be of crucial importance in this process.

#### P06.144

#### Regulation of the level of a natural autoantibody, anti-HSP70 by the 8.1 ancestral haplotype in patients with colorectal cancer

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There are only scarce data on genetic regulation of natural autoantibody production in healthy individuals. Recently we published papers indicating that levels of some natural autoantibodies is regulated by the IL-6 gene and/or the HLA-DR15 and -DR16 antigens. According to our previous findings the most frequent ancestral extended haplotype (AH8.1) is a strong risk factor for colorectal cancer. Therefore we decided to compare serum concentration of an IgG type natural autoantibody anti-HSP70 in carriers and non carriers of the AH8.1 in cancer patients. Commercial ELISA kit was used for measuring serum levels of the anti-HSP70 autoantibodies. Carriers of the RAGE -429C, HSP70 1267G, TNF -308A and LTA 252G alleles and C4A\*Q0 genotype were considered as AH8.1 carriers. The study was performed in 188 patients with colorectal cancer and 203 healthy controls. Anti-HSP70 levels were significantly ( $p<0.001$ ) higher in the patients than in the controls. In the patients' group AH8.1 carriers had significantly

( $p=0.008$ ) higher anti-HSP70 levels than the non-carriers, while no significant difference was observed in the controls. The AH8.1 carriers had a significantly higher chance to have high (in the highest quartile  $>1000$  AU/ml) anti-HSP70 serum concentration (age and sex-adjusted OR: 3.21 (1.24-8.51),  $p=0.016$ ). Interestingly enough this association could be detected only in the 105 male patients (age-adjusted OR: 17.1 (2.8-103.9) but not in the 83 female patients (OR: 1.18 (0.33-4.22). These observations indicate that the serum concentration of the autoantibodies to HSP70 is under genetic influence in patients with colorectal cancer, this association is, however, restricted to men.

#### P06.145

#### A frequent germline EPCAM deletion causes Lynch syndrome by hypermethylation of the MSH2 promoter

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Recently it was shown that Lynch syndrome can be caused by germline hypermethylation of the MSH2 promoter. In addition, it has been demonstrated very recently that germline deletions of the 3' region of the EPCAM gene cause transcriptional read-through of EPCAM which results in silencing of MSH2 by hypermethylation. We wanted to determine the prevalence of germline and somatic MSH2 promoter hypermethylation in a large group of Lynch syndrome-suspected patients. From a group of 333 Lynch syndrome-suspected patients who had been diagnosed with colorectal and/or endometrial cancer, we selected those, who had no MLH1, MSH2 or MSH6 germline mutation and who lacked MSH2 protein staining in their tumours, or, if staining was unavailable, had tumours with microsatellite instability. Methylation assays were performed to test the patients for germline MSH2 promoter hypermethylation. A subset of patients with MSH2-negative tumours were analysed for somatic MSH2 promoter hypermethylation and subsequently for EPCAM deletions.

Thirty-six patients were screened for germline MSH2 promoter hypermethylation and tested negative. However, three of the eleven patients with loss of MSH2 protein in their tumours had somatic MSH2 promoter hypermethylation in their tumour, which was caused by a germline EPCAM deletion.

The prevalence of somatic MSH2 promoter hypermethylation caused by germline EPCAM deletions in our selected patient group is 8%. Extrapolated to the group of 333 Lynch syndrome-suspected patients the frequency is 0.9%. More importantly, this deletion accounts for a substantial proportion (9.4%) of the genetically proven Lynch syndrome cancer cases in our cohort of 333 Lynch syndrome-suspected patients.

#### P06.146

#### Involvement of MSH6 germline mutations in HNPCC Brazilian families

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Hereditary non-polyposis colorectal cancer syndrome (HNPCC) is associated with malfunction of postreplicative mismatch repair (MMR). While the MMR genes MSH2 and MLH1 account for a majority of HNPCC cases, the involvement of the MSH6 gene is continually rising. Altogether, MSH6 mutations account for 5-10% of kindreds in which MSH2 and MLH1 mutations are excluded. In contrast to HNPCC families linked to MSH2 or MLH1 mutations, the families associated with MSH6 mutations often display diverse and less typical clinical features, such as early age at onset and high microsatellite instability (MSI). Therefore, MSH6 mutation families are less likely to fulfil diagnostic criteria such as the Amsterdam II criteria (AC II) and the revised Bethesda guidelines (rBG), and are being underdiagnosed. The aim of the present study was to evaluate the contribution of MSH6 germline mutations in brazilian families with suspected Lynch syndrome. 38 unrelated families (twelve AC I, II and twenty-six rBG) without MLH1

and MSH2 germline mutation were included in this study. All coding regions and intron-exon boundaries of MSH6 gene were completely analysed using direct sequence analysis. We detected 5 MSH6 mutation (4 missense and 1 protein-truncating mutations). Although, missense variants are labeled as doubtfully pathogenic, clinical data display a great resemblance between missense-variant carriers and truncating mutation carriers. We conclude that, in all patients suspected to have HNPCC, MSH6 mutation analysis should be considered.

#### P06.147

#### Evaluation of MLH1 and MSH2 gene mutations in a subset of Iranian families with hereditary nonpolyposis colorectal cancer (HNPCC)

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Hereditary nonpolyposis colorectal cancer is the most common form of hereditary colorectal cancers accounting for 5 to 10% of all colon carcinoma. It is inherited in an autosomal dominant mode and caused by germline mutations in mismatch repair genes (MMR) chiefly MLH1 and MSH2.

The lifetime risk of colon cancer in affected persons is 80%. Screening, prevention strategies and consequently treatment options will be improved by understanding of the genetic basis of this disorder. The aim of this study was to assess mutations in MLH1 and MSH2 genes in a subset of Iranian HNPCC patients.

The families that fulfill Amsterdam criteria were selected as HNPCC families. Genomic DNA was extracted from the peripheral blood of the samples and germline mutations of MLH1 and MSH2 were detected by PCR-single strand conformation polymorphism (PCR-SSCP) and DNA sequencing techniques.

Germline mutations were found in 20 cases. Of these mutations, 14 were found in MLH1 and 6 in MSH2 genes thus MLH1 gene had higher mutation rate than MSH2. Eighteen out of 20 detected mutations in our population were previously reported and two were novel.

Our results demonstrated that mutation range as well as genes involved in HNPCC is different from one region to other and characterizing mutations could be very helpful in diagnosis of the at risk individuals.

#### P06.148

#### Screening for germline mutations of MLH1, MSH2, MSH6 and PMS2 genes in Slovenian colorectal cancer

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Microsatellite instability (MSI) is present in more than 90% of hereditary non-polyposis colorectal cancer (HNPCC) cases, and is therefore a feasible marker for the disease. Mutations in MLH1, MSH2, MSH6 and PMS2, which are one of the main causes of deficient mismatch repair and subsequent MSI, have been linked to the disease.

In order to establish the role of each of the 4 genes in Slovenian HNPCC patients, we performed MSI analysis on 938 unselected CRC patients and subsequently searched for the presence of point mutations, larger genomic rearrangements and MLH1 promoter hypermethylation in patients with MSI-high tumours.

We detected 68 (7.2%) patients with MSI-H tumours, of which 13 patients (1.4%) harboured germline defects: 7 in MLH1, 5 in MSH2, 1 in PMS2 and none in MSH6. Twenty-nine germline sequence variations of unknown significance and 17 deleterious somatic mutations were found. MLH1 promoter methylation was detected in 56% of patients without detected germline defects and in 1 suspected HNPCC.

Due to the specific absence of germline defects in MSH6, we adapted the HNPCC detection strategy for the Slovenian population of CRC patients, whereby germline alterations should be first sought in MLH1 and MSH2 followed by a search for larger genomic rearrangements and PMS2 mutations, and when no germline defects are found, mutation analysis of the MSH6 gene should be performed. Our study demonstrates that the incidence of MMR mutations in a population should be known prior to the application of one of several suggested strategies for detection of HNPCC.

#### P06.149

#### Molecular characterization and screening of mismatch repair genes mutations in 667 Spanish patients with a familial form of nonpolyposis colorectal cancer

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Introduction: Familial aggregation of colorectal cancer (CRC) is estimated to be approximately 15-20% of all CRC. The autosomal dominant Lynch syndrome (LS) is the most common hereditary CRC (2-7% of all cases). Lynch tumours show microsatellite instability (MSI) due to aberrant DNA mismatch repair (MMR). The genetic causes are mutations in MLH1, MSH2, MSH6 and PMS2.

Aims: To characterize the molecular basis of CRC aggregation and determine the minimal frequency of LS in a series of Spanish families.

Samples and methods: We studied 667 tumour samples belonging to patients with a suspect of a familial form of CRC and collected clinical and familial data of the index patients and pathological data of tumours. MSI and/or immunohistochemical expression of MMR proteins was assessed. In cases with MSI and/or lack of expression of at least one MMR protein, we screened MLH1, MSH2 and MSH6 genes in peripheral blood samples for both point mutations and great rearrangements.

Results: 182 cases (27.3%) showed MSI and/or lack of the expression of MMR proteins. From these cases we have identified 66 families (36.2%) with alteration in one MMR gene (Table 1).

	MLH1	MSH2	MSH6
Deleterious point mutation	30	19	2
Great rearrangement	1	5	
Unknown significant variant	5	3	1
Epimutation	1		

Table 1. Distribution of MMR changes

Comments: Beside these cases, we identified 26 additional families that fulfilled the clinical and molecular criteria of Familial CRC Type X.

Type of mutations and the correlation with clinical and familial data will be presented in this series of families.

#### P06.150

#### Family history compared to age as indicator for MSI testing results in a comparable positive predictive value for Lynch syndrome

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Introduction: Most colorectal tumours that are due to Lynch syndrome show high microsatellite instability (MSI-high). Clinical geneticists use MSI tests when a family history is suspected for Lynch syndrome. To increase the detection of Lynch syndrome, also pathologists select patients with colorectal or endometrial cancer for MSI testing in case they are diagnosed younger than 50 years or have a second Lynch associated cancer younger than 70 years (Gut 2005).

Methods: Index patients under suspicion for Lynch syndrome by a clinical geneticist (n=885) or a pathologist (n=406) were tested for MSI. When MSI-high, these patients could opt for germline mutation analysis in MLH1, MSH2, MSH6 and/or PMS2.

Results: Comparison of the group of patients under suspicion for Lynch syndrome by clinical geneticists or pathologists showed a comparable mean age at diagnosis of  $49 \pm 12$  versus  $48 \pm 13$  years ( $p=0.7$ ), the percentage of MSI tests performed in a colorectal cancer (others mainly endometrial cancer) was 77% versus 96% ( $p<0.001$ ), and right sided colon cancer 69% versus 65% ( $p=0.9$ ). MSI-high was present in 16% (144/885) and 18% (74/406) of the tests ( $p=0.38$ ). MSI-high tumors were explained by the presence of a germline mutation in 58% and 40% ( $p=0.09$ ), and by hypermethylation of the MLH1-promoter in 21% and 26% respectively.

Conclusion: These results underscore that family history taking continues to be important for the detection of Lynch syndrome, but that MSI testing based on age only is an excellent alternative, reducing workload for clinical geneticists substantially.

## P06.151

## Cancer risks associated with germline MMR gene mutations: results from a multi-center French study

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Lynch syndrome is an autosomal dominant disorder caused by germline mutations of mismatch repair (MMR) genes: *MLH1*, *MSH2* and *MSH6*. Mutation carriers are at high risk for colorectal and endometrial cancers, and other rarer localisations: small bowel, urologic and biliary tract, ovary and stomach.

This study aimed to estimate precisely the risk for different tumors in a large sample of French families with Lynch syndrome.

A total of 507 pedigrees with a MMR gene mutation (238 *MLH1*, 239 *MSH2*, 30 *MSH6*) and 9834 informative members were ascertained from 40 cancer family clinics in France. Cancer risks were estimated using the GRL method, based on maximum likelihood that corrected for ascertainment by conditioning on all observed phenotypes.

The cumulative risks of colorectal cancer by age 70 years were 35.2% (95% CI: 24.7-51.3) for men and 31.5% (19.8-50.5) for women; significant risk differences were found between mutated genes ( $p < 0.01$ ). *MLH1* and *MSH2* mutation carriers were at similar risk for colorectal cancer whereas *MSH6* carriers were at markedly lower risk. Risks of endometrial and ovarian cancer were respectively 10.8% (7.6-14.3) and 4.1% (2.7-5.5). Estimations for other localisations did not exceed 2%.

Our results provide evidence for lower risk of colorectal cancer than previously published, particularly for *MSH6* carriers, but also lower risk of endometrial cancer in Lynch syndrome. Their impact on the counselling and management of mutated MMR gene carriers should be taken into account.

## P06.152

Novel *MSH6* mutations in HNPCC families with endometrial cancer.

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Introduction: Families with germline *MSH6* mutations have also shown different clinical features compared with traditional HNPCC phenotype, such as later age of cancer diagnosis, lower penetrance, and predominance of endometrial carcinomas.

For women, colorectal cancer risk was significantly lower and endometrial cancer risk significantly higher in *MSH6* carriers compared with *MLH1* and *MSH2* carriers.

Objective: To determine the prevalence of *MSH6* (a mismatch repair gene) mutations in a cohort of HNPCC families with endometrial cancer patients.

Patients and Methods: A cohort of 20 HNPCC families who were known to the Regional Medical Genetic Counselling Unit.

Molecular analysis of DNA in all participants for mutations in *MSH6* by HA-CAE technique and sequencing analysis.

Results: A novel truncating mutation in *MSH6* was identified in a woman with breast and endometrial cancer. A novel missense mutation was identified in two families. No genomic rearrangements in *MSH6* were identified.

Conclusion: *MSH6* mutations are more common in HNPCC families with endometrial cancer history. Genomic rearrangements do not contribute to a significant proportion of mutations in *MSH6*, but missense variants are relatively common and their pathogenicity can be uncertain.

## P06.153

*MUTYH* associated polyposis - variable clinical phenotype and report on novel mutations

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To further characterise 215 APC mutation-negative patients with colorectal neoplasias classified in classical, attenuated, or atypical forms of familial adenomatous polyposis coli (FAP) we performed mutation screening in the *human Mut Y homologue (MUTYH)* gene. The incidence of *MUTYH* mutations was 15% for biallelic and 3.7% for monoallelic mutations.

We found six novel *MUTYH* mutations and two novel monoallelic missense mutations of unclassified pathogenicity in attenuated FAP (AFAP) patients.

Most of the *MUTYH*-associated polyposis coli (MAP) patients (57%) were AFAP patients as expected (19/33), but 10% (3/33) MAP patients displaying early-onset classical FAP and 18% (6/33) patients with only few adenomas at higher age. Biallelic cases had a high incidence of extracolonic polyposis in 32% (9/25 informative) and colorectal cancer (CRC) in 33% (11/33) of the cases.

The clinical picture of MAP ranged from classical FAP or synchronous colorectal cancer (CRC) at age 30 years to few adenomas at age 54 years without evidence of CRC, initially suspected for hereditary non-polyposis colorectal cancer (HNPCC). The mean age of onset was 43 years, with 11 (33%) patients being younger than 40 years of age, indicating that the clinical manifestation can be earlier than so far reported. Monoallelic *MUTYH* mutation carriers had a positive family history in seven of eight cases (86%) overlapping with HNPCC type X. This allows the hypotheses that monoallelic *MUTYH* mutations might 1) act as low-penetrance CRC susceptibility modifier and/or 2) cooperate in a disease-causing synergism with mutations in other gene involved e.g. in the BER pathway.

## P06.154

Identification of *p53* gene tandem mutation and effect of its surrounding sequences during development of colon cancer

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Mutation in *p53* tumor suppressor gene is highly frequent in different cancers. The *p53* protein is considered as a key combination in counteracting stress messages such as DNA damage. Prevalence of somatic mutations in *p53* gene has been reported in many tumors, and hereditary mutations in this gene increase the chance of developing a widerange of cancers. Colorectal cancer is the third cause of the cancer-related death in the world.

The aim of the study is to determine *p53* gene mutation in developing colon cancer among Iranian patients. To understand of the etiology of colon cancer in Iran, we initiated a study to investigate *p53* gene mutations among Iranian colon cancer patients.

32 Formalin-fixed, paraffin-embedded colon cancerous tissues with adenocarcinoma diagnosis (after colectomy) and normal tissue adjacent to tumor were collected for analysis from cancer patients in the Cancer Institute of Tehran. No patient had been given chemotherapy or radiotherapy before the operation. The *p53* gene mutations were determined by direct DNA sequencing after DNA extraction and doing PCR for each sample. We found a tandem mutation at codon 168 (CAC>TAC then TAC>TGC) in tumor tissue. In previous study, we showed that surrounding sequences of mutated *p53* gene codons (5'-GT and 5'-GG sequences) are high. In this investigation, our finding suggested that 5'-GT and 5'-GG surrounding sequences of the mutated *p53* gene codons may play an important role in developing the *p53* gene modification and mutation. This hypothesis needs *in vitro* investigation for confirmation.

## P06.155

## New roles for pharmacogenetics in metastatic colorectal cancer

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The epidermal growth factor receptor (EGFR) plays an important role in tumorigenesis of colorectal cancer. Anti-EGFR monoclonal antibodies have been shown to be helpful in the treatment of patients with metastatic CRC (mCRC). Recent data indicate that kRAS mutations are an independent, negative, predictive marker for response to anti-EGFR agents.

The aim of this study is to compare sequencing analysis and a commercial kit for kRAS mutation detection.

Genomic DNA was extracted from archival tumour sections of 27 patients affected by mCRC.

KRAS analysis was carried out by sequencing of exon 2 and real-time PCR (commercial kit) to identify somatic mutations located in codons 12 and 13 (Gly12Asp, Gly12Ala, Gly12Val, Gly12Ser, Gly12Arg, Gly12Cys, and Gly13Asp). In 2 cases DNA was poor and both methods failed, probably due to old-aged. We found 10 mutated cases (10/25, 40%): 5 mutations detected by both methods, 3 samples not analysable by commercial kit and 2 cases mutated for sequencing alone (both Gly13Asp). The remaining 15 cases resulted wt by sequencing and 6 of these were not analysable by commercial kit (6/15, 40%). Our results show that sequencing is the most reliable technique to detect kRAS mutations. The commercial kit has an elevate rate of failure (48%) and gives two false negative results. kRAS mutation status might allow the identification of patients who are likely to benefit from anti-EGFR agents and avoid a costly and potentially toxic administration of this treatment in nonresponder patients, that can be treated with a different therapy.

## P06.156

Two novel variants of the *PML* gene in breast and colon cancer patients

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The *PML* (promyelocytic leukemia) gene is an important tumor suppressor, that encodes the PML protein. The PML protein is concentrated in special subnuclear structures, the so-called PML nuclear domains and it plays a role in the formation and stability of these domains. PML harmonically coordinates and controls transcription, antiviral response, DNA damage repair, senescence, induction of apoptosis and growth arrest. We were testing the hypothesis, that germline disruption of the *PML* gene may predispose to an increased risk of the cancer development. We used direct sequencing for mutation screening in 17 colon and 22 breast cancer patients. We have found, among others, two novel single nucleotide substitutions, c.83C>T (p.T28I) within exon 1 in a 42-years old female breast cancer patient and c.1558C>T (p.P520S) within exon 6 in a 32-years old male colon cancer patient. We have performed a population study in 100 and 214 non-cancer patients, respectively, using the RT-PCR method, in order to detect the frequency of these novel variants in general population. None of the variants was found in any non-cancer patients. In conclusion, we have found two rare novel missense variants in the *PML* gene. Their pathogenicity is uncertain, however, with respect to fact, that they were found only in cancer patients, they may be associated with an increased risk of the cancer.

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## P06.157

*PMS2-PMS2CL*-"hybrid"-alleles containing pseudogene-specific sequence variants have a high prevalence but no apparent functional effect on colorectal cancer susceptibility

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*PMS2* and its pseudogene *PMS2CL*, share paralogous exons 9 and 11-15 and are embedded in an inverted duplication on chromosome 7p22. Sequence exchange between dupicons may lead to functional *PMS2*-"hybrid"-alleles containing *PMS2*-specific sequence variants at the 5'- and *PMS2CL*-specific variants at the 3'-ends. The reference sequences thus cannot be relied upon to distinguish between gene and pseudogene. We previously reported an RNA-based assay that allows reliable mutation analysis and unequivocal identification of *PMS2*-"hybrid"-alleles. We now found that overall "hybrid" alleles account for one third of 300 *PMS2* alleles in control individuals. Depending on the population 14-60% of "hybrid"-alleles carry *PMS2CL*-specific sequences in exons 13-15, the remainder only in exon 15. Analysis of associated polymorphisms revealed that exons 13-15 "hybrid"-alleles constitute four different haplotypes but involve the same breakpoint and appear to trace back to a single ancient founder event. We identified one sequence variant specific for all exons 13-15 "hybrid"-allele and developed a simple gDNA-PCR assay that can be used to identify carriers of "hybrid"-alleles with high sensitivity and specificity (100% and 98%, respectively). This test may render *PMS2* mutation analysis in diagnostic laboratories more reliable. Exon 13-15 "hybrid"-alleles harbour a missense variant of so far unknown functional significance, and we used the novel assay to determine "hybrid"-allele carrier frequency in colorectal cancer patients. We found no significant difference in allele frequencies between patients and controls, indicating that the missense variant is unlikely to play a major role with regard to colorectal cancer susceptibility.

## P06.158

## Case report of CRC family with two substitutions in APC gene

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Familial adenomatous polyposis (FAP) is an autosomal dominant inherited disease characterized by the presence of hundreds to thousands colorectal adenomatous polyps and several clinical manifestations. FAP is known as a colorectal cancer predisposition syndrome and is caused by a mutation in the tumor-suppressor APC gene which often leads to creation of truncated APC protein.

In this report we demonstrate case of 3 generation family from our group of CRC patients. Proband, 30-year old woman, was involved in extensive colorectal carcinoma and after detailed clinical examination and pedigree assembly it was confirmed FAP diagnosis. Subsequently it was performed analysis of APC gene with detection of 2 substitutions - causal mutation c.2413C>T (p.Arg805X) and mutation c.7504G>A (p.Gly2502Ser) with unexplained influence on FAP genesis.

It occurs comparatively a lot of relatives with various diagnosis of cancer in the proband's family and there are also 7 children in risk. Our aim was to make APC gene analysis of this children and other living proband's relatives to determine whether this substitutions occur on one allele of APC gene together or on two alleles separately. This molecular results are important for determination of the FAP manifestation risk and for selection of family members for preventive oncological monitoring.

## P06.159

## Zoom-in CGH-array to better characterize germline large rearrangements involving APC gene in FAP French families

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Familial Adenomatous Polyposis is caused by germline mutations in the APC gene. Most mutations are point mutations or small insertions/deletions. Six to 12% of FAP patients carry a large deletion within the APC gene, which encompass one to all exons. Until now, most articles reported large deletions without precisely characterize the breakpoints and/or the mechanism. Here, we aimed to assess the proportion of

germline APC deletions in the French patients affected with classic FAP, and to characterize the different breakpoints. All samples were hybridized in a 244k oligonucleotide CGH-arrays. In parallel, we also used a dedicated high-resolution oligonucleotide CGH array to exactly size the deletions. We screened 126 patients with polyposis without any point mutation in the APC gene by using Multiplex Ligation-dependent Probe, and were able to detect 62 large rearrangements (8% of all deleterious mutations). No rearrangement was recurrently found. In the very large deletions with breakpoints outside the APC gene, the size ranged from 50kb to 17Mb. We found 7 hot regions with more than 2 breakpoints within 30kb. Finally, we characterized a new large rearrangement with an insertion-deletion. High-resolution oligonucleotide array-CGH showed clearly its interest in the characterization of large rearrangements of the APC gene. Screening for APC mutations in FAP patients should benefit of this technique to identify precisely the origin and better understand the underlying molecular mechanisms.

#### P06.160

##### A remarkable APC mosaicism with two mutant alleles

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It has recently been estimated that APC mosaicism accounts for 11% of the sporadic cases of Familial Adenomatous Polyposis (FAP). We report a remarkable APC mosaicism characterized by the presence of two mutant alleles. The index case presented a typical form of FAP diagnosed at age 29. APC sequencing revealed a heterozygous deleterious mutation within exon 15 (c.2099del, p.Asp700AlafsX18). This mutation was inherited from his father who presented an attenuated form of FAP diagnosed at age 54. Analysis of APC in an index case's brother detected, unexpectedly, another mutation of unknown biological significance, affecting the same nucleotide (c.2099A>C, p.Asp700Ala). Haplotype analysis showed that both mutations were on the same paternal allele. In the index case's father, specific analysis of the c.2099 nucleotide by SNaPshot allowed us to detect the wild type and both mutant alleles (c.2099del and c.2099A>C) in lymphocytes, normal colorectal tissue, adenoma, adenocarcinoma, normal liver tissue and liver metastasis, indicating therefore a remarkable mosaicism affecting endoderm and mesoderm derivatives. The presence of these two distinct alterations at the same position might be explained by two hypotheses: The first one is an early post-zygotic mutation (c.2099del or c.2099A>C) followed by an incorrect repair (c.2099A>C or c.2099del, respectively); the second one is a *de novo* pre-zygotic deleterious mutational event (c.2099del) in an index case's paternal grand-parent, followed by an incorrect post-zygotic reversion (c.2099A>C) in the index case's father. Since sequencing and SNaPshot analyses showed that the c.2099del mutation is the predominant mutation, this second hypothesis is the preferred one.

#### P06.161

##### APC gene mutation status in Polish FAP patients

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Familial adenomatous polyposis (FAP) is hereditary predisposition to occurrence numerous of polyps in the colon and rectum. There is a high heterogeneity with regard to the number and time of the occurrence of polyps. FAP is associated with mutations in the APC tumour suppressor gene, which was described in 1991. Since then, many studies have been done to analyse the distribution of mutations in individual populations and to determine the function of the gene and a diagnostic approach to FAP. Here the APC gene was studied with respect to the occurrence of small mutations and large rearrangements in 300 unrelated Polish FAP families. Ninety-seven mutations were identified in 164 families. Out of these mutations, 80 were small mutations, including 58 small mutations that were first identified in the Polish population (42 novel and 16 described previously). An increased frequency of mutation c.3927\_3931delAAAGA was observed in 10%

of the Polish group. Seventeen large rearrangements were found in 29 families. Out of those rearrangements, 8 repeat rearrangements occurred in 20 families. A problem in fast molecular diagnostics of FAP is a high heterogeneity of mutations in the APC gene. It seems that a multiplex ligation-dependent probe amplification test and searching for small mutations by the use of screening methods at the 5' end of exon 15 and exons 14, 9, 11, 13, 5, and 3, help to improve the molecular diagnostics of FAP in Polish patients.

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#### P06.162

##### APC gene mutations in Iranian patients with familial adenomatous polyposis (FAP)

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**Background:** Familial adenomatous polyposis (FAP) is an autosomal dominant syndrome characterized by development of hundreds to thousands of adenomatous polyps in the colon. In 80-90% of the cases of classic FAP, mutations in the adenomatous polyposis coli (APC) gene is the cause underlying the syndrome. The molecular epidemiology of the APC gene mutations has not been investigated among the Iranian patients. In this study we will report on the first systematic investigation of the APC gene mutations.

**Methods:** Patients with FAP were enrolled into this study through referral to our genetic counseling clinic. For genetic tests genomic DNA was extracted from the 10ml of peripheral blood. Specific primers were used to amplify coding regions of the APC gene. Amplicons was bidirectionally sequenced.

**Results:** 25 probands was registered with the mean age of diagnosis 28.91 and 64% male and 36% female. 52% had an autosomal dominant pattern of inheritance and 48% had negative familial history. 3 nonsense, 3 missense and 2 sense mutations were found. The nonsense mutations were Q264X, Q1303X, S1315X and Q264X. The missense mutations were V1822D, E1317Q, N944I and sense mutations were G1678G and T1493T.

**Conclusion:** APC mutations are found among the FAP families in Iran. The complete mutational investigation of these families helps in pre-clinical diagnosis of unaffected family members and may lead to potential founder mutations underlying this disease in Iran.

#### P06.163

##### Molecular diagnostics in the Czech FAP families

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Familial adenomatous polyposis (FAP) is autosomal dominant syndrome associated with germline APC mutation with almost 100% risk of colorectal cancer. The typical FAP is characterized by hundreds to thousands of colorectal adenomatous polyps and by extracolonic manifestations. An attenuated FAP (AFAP) is characterized by less than 100 adenomas and later onset of the disease. The mutations in *MUTYH* gene leads to *MUTYH* associated polyposis (MAP). MAP is autosomal recessive form of polyposis with manifestation similar to AFAP.

We analyzed the APC gene for germline mutations in 340 FAP/AFAP patients. Mutation screening was performed using DGGE. DNA fragments showing an aberrant electrophoretic banding pattern were sequenced. In addition, all APC-mutation-negative probands were screened for large deletions of the APC gene using multiplex ligation dependent probe amplification (MLPA).

We identified 80 germline mutations among 126 unrelated probands including large deletions. Nine germline APC mutations detected last year have not been reported yet, which gives evidence of great variability of mutations.

We examined the whole *MUTYH* gene in 120 APC-mutation-negative probands, thereto we screened for mutations in the exon 7 and 13 of *MUTYH* gene in 72 APC-mutation-negative probands. Mutation

screening was performed using denaturing high performance liquid chromatography (dHPLC) or high resolution melting (HRM). Samples showing unique profiles were sequenced.

We detected 2 patients with biallelic mutation in *MUTYH* and 6 patients with monoallelic *MUTYH* mutation. Now we have started to test the presence of *MSH6* mutation in carriers of monoallelic *MUTYH* mutation.

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#### P06.164

##### Breakpoint identification of large STK11 germline deletions in Peutz-Jeghers syndrome patients using fine-tiling CGH arrays

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Peutz-Jeghers syndrome (PJS) is an autosomal dominantly inherited cancer predisposition syndrome characterized by the presence of hamartomatous polyps, mucocutaneous pigmentation, and an increased risk for malignancies of the colorectum, stomach, pancreas, breast, and ovaries. It is caused by germline mutations in the serine/threonine protein kinase 11 (STK11, also called LKB1) tumour suppressor gene. Germline STK11 mutations can be identified in 80 to 94% of PJS patients when combining a screening for point mutations and large deletions using direct sequencing and multiplex ligation-dependent probe amplification (MLPA), respectively. With the aim to identify large STK11 deletions and simultaneously delineate the breakpoints with high precision (approx. 100-200bp), we designed a custom fine-tiling CGH array with a median probe spacing of 10bp covering the entire STK11 gene including 135 kb of genomic sequence up- and downstream of the gene, and applied the technique on 5 STK11 deletion carriers previously identified by MLPA as well as on 2 mutation-negative PJS patients. We will report on the overall accuracy of the fine-tiling CGH array in fine-mapping the breakpoints and thus enabling cost- and time-saving carrier screening of at risk-family members by subsequent PCR-based fragment length analysis/direct sequencing.

#### P06.165

##### Germline mutations of the STK11 gene and clinical findings in Czech Peutz-Jeghers families

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Peutz-Jeghers syndrome (PJS) is an autosomal dominant inherited disorder characterized by mucocutaneous hyperpigmentation and gastrointestinal hamartomatous polyposis. PJS patients have increased risk of developing cancer over the general population, predominantly in gastrointestinal tract. Germline mutations in the serine/threonine kinase 11 (STK11) gene have been found to be responsible for the disease. Here we report clinical findings and molecular analysis of 9 individuals from 6 Czech families. We analyzed promotor and the entire coding region including the splice-site boundaries of the STK11 gene in genomic DNA of the probands by sequencing analysis and multiplex ligation probe-dependent amplification (MLPA) assay. By direct sequencing of the STK11 gene, we identified two frameshift mutations (c.350dupT and c.589\_597+2del11) in 3 individuals from two families. The remaining 6 patients were examined by MLPA method and 4 patients from two families harboured large deletions (c.-277-?\_290+?del and c.-1114-?\_1365+?del). No mutation was identified in two patients with sporadic disease. In conclusion, we found germline mutations in five familial and two sporadic cases. One patient with frameshift mutation (c.350dupT) and severe phenotype died due to gastric cancer at her 29, the other probands were without any malignancy up to date. This is the first report dealing with PJS patients in Czech Republic.

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#### P06.166

##### Interphase FISH detection of prognostically important chromosomal aberrations in B-CLL

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Chromosomal aberrations are independent prognostic factors in B-cell chronic lymphocytic leukemia (B-CLL). Cytogenetic abnormalities identify groups of patients with different time to progression and overall survival.

There are two main risk groups recognized. Low-risk patients with normal karyotype or 13q deletion as a sole abnormality have excellent prognosis. High-risk patients with 11q deletion and particularly 17p deletion do not respond to conventional therapy and tend to have a rapidly evolving disease. Prognosis of patients with trisomy 12 is intermediate.

Interphase fluorescence in situ hybridization (I-FISH) is currently preferred molecular-cytogenetic method for identification of the chromosome aberrations in B-CLL.

The aim of this study was the detection of prognostically important chromosomal aberrations in B-CLL patients and comparison of their prognostic significance with the other biological and clinical factors.

We performed I-FISH analysis using a DNA probe set to detect deletions 17p, 11q, 13q, trisomy 12 and translocations involving 14q32 on bone marrow and peripheral blood samples of 480 B-CLL patients.

Chromosomal aberrations analyzed by I-FISH were found in 73% of patients including 54% with single aberration, 17% with two and 2% with three or more aberrations. The most frequent aberration was 13q14 deletion found in 39% of patients, followed by 11q23 deletion in 12%, trisomy 12 in 11%, and 17p13 deletion in 9% of patients. Rearrangement involving 14q32 was found in 1% of patients. Correlations of I-FISH results and some clinical and laboratory findings will be presented.

#### P06.167

##### Array CGH identifies potential candidate genes in AML without recurrent molecular and cytogenetic aberrations

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Introduction: Chromosomal and molecular aberrations are an integral part of AML classification and influence prognosis and therapy. Nevertheless AML with none of the classical genetic changes do exist and one can assume other genetic events in these cases leading to different expression of the disease. Therefore we analyzed AML without recurrent aberrations for possible candidate genes by array CGH.

Methods: Bone marrow from cytological and immunophenotypical confirmed AML patients was analyzed by conventional cytogenetics (GTG-Banding), FISH and PCR methods for typical aberrations indicated by WHO. DNA from inconspicuous samples was hybridized on Affymetrix SNP 6.0 Arrays featuring 1.8 million markers, SNPs and CNVs (copy number variations), one half each. The majority of gains and losses detected by Affymetrix genotyping console 3.0 were CNVs and excluded in our study.

Results: About two thirds of the AML samples show at least one domain carrying a potential tumor associated gene. These include, for example, ZBTB16, IL1R2 or EPS8, which are important for regulation of apoptosis, cell cycle progression, and signal transduction. Chromosomal changes leading to the deregulation of the involved pathways may potentially be causative for the development of AML.

Conclusions: Array CGH seems to be a potent method for the identification of new candidate genes in AML without recurrent cytogenetic and molecular aberrations.

**P06.168****Characterization of a novel ETV6-NTRK3 Fusion Transcript in Acute Myeloid Leukemia**

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Introduction: ETV6 transcription factor is frequently rearranged in diverse tumors forming a variety of fusion genes. We could identify a novel ETV6-NTRK3 gene fusion in an AML patient.

Methods: AML was diagnosed morphologically and by flow cytometry. Conventional karyotyping demonstrated a t(12;15)(p13;q25) confirmed by whole chromosome paints. ETV6 break apart FISH proved an involvement of this gene. 3'RACE-PCR was performed to characterize the unknown 3'end of the fusion product. PCR products were subsequently cloned and sequenced. The rearrangement was verified by fusion-specific reverse transcriptase PCR.

Results: RACE-PCR and sequencing analysis identified NTRK3 (neurotrophic tyrosine receptor kinase 3) as in-frame fusion partner of the ETV6 gene. NTRK3 transcripts encode either catalytically active proteins or truncated isoforms that lack the intracellular kinase domain. The ETV6-NTRK3 transcript fuses ETV6 exons 1 through 5 with exons 13b and 14b of a truncated NTRK3 isoform and encodes a protein that contains the amino-terminal HLH domain of the ETV6 protein and C-terminal amino acids 529-612 of the NTRK3 protein.

Conclusions: We identified a novel ETV6-NTRK3 gene fusion in a patient with minimal differentiated AML. Interestingly, this chromosomal rearrangement is documented in a significant fraction of patients with secretory breast cancer and congenital fibrosarcoma.

**P06.169****Sibs' birth defects in families with acute leukemia**

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Clinical and genealogical analysis was conducted in 240 families of the Lviv region (Ukraine) which had two and more children. The basic group was made with 120 families, which had children with acute leukemia. Children were treated in hematology department of the Lviv Regional children's specialized clinical hospital 1994 - 2008 concerning. The age of children was from 3 months till 16 years (3,8±1,9 years).

Research carried by a method "case - control". Among 120 families of the basic group, which had the two and more children, we observed 11 families (9,2 %), where, except for cancer at proband, sibs had birth defects. In control group the birth defects at sibs met in 3 (2,5%) cases (P<0,05).

Five from among 11 families, in which sibs suffered from BD and leukemia, had two children at the moment of research, and both children were ill with the following diseases: Down's (trisomy 21) syndrome, cryptorchidism, congenital heart disease and leukemia (three families), bilateral hydronephrosis. The control group showed BD in two male junior sibs (congenital dislocation of left hip joint and cryptorchidism) and in one female senior sib (cleft lip).

More often the birth defects were observed at the senior brothers and sisters, which did not coincide on localization with cancer in proband. The increase of BD rate among the sibs of proband having malignant tumors can be explained by the combined realization of teratogenic, oncogenic and mutagenic effects upon the action of various environmental factors.

**P06.170****Chromosomal abnormalities detected with FISH in the patients with B-chronic lymphocytic leukemia**

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We have analyzed the frequency of genomic aberrations in a series of 44 B-cell chronic lymphocytic leukemia (B-CLL) patients diagnosed in our institution, in order to evaluate their prognostic implications. The frequency and type of aberrations were examined by interphase fluorescence *in situ* hybridization technique, using Vysis DNA probes for detecting deletions in 11q22.3, 13q14.3, 13q34 and 17p13.1 regions, and trisomy 12. Our results showed 63.6% of patients with clonal chromosomal alterations. The most frequently detected aberration was

the 13q14.3 deletion (48%), followed by 11q22.3 deletion (11%) and trisomy 12 and 17p13.1 deletion (5%, both). Deletion of 13q34 was observed in one patient (2%) and was accompanied with monosomy 12, untypical change for CLL. In this group, trisomy 12 was not observed as a separate aberration - it was seen only in coexistence with 13q14.3 deletion. Moreover, deletion 13q14.3 was frequently associated with another genetic aberrations (11q22.3, 17p13.1). Molecular-cytogenetic findings were correlate with disease status (stable versus progressive), Rai stage and CD38 expression and other clinical and laboratory parameters. This results will be presented in the future. This is the first evaluation of cytogenetic alterations in the patients with CLL in our country, showing the presence of typical but also rare and untypical chromosomal changes.

**P06.171****BCR-ABL rearrangement and 'variant' Philadelphia chromosome in Chronic myeloid leukemia**

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Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the presence of the Philadelphia chromosome (Ph) in about 95% of patients (pts). Ph is a result of reciprocal translocation between the long arms of chromosomes 9 and 22 [t(9;22)(q34;q11)]. About 5% of Ph-positive CML pts have variant translocations (vPh) which can be divided into a "simple" (22q11 and one additional breakpoint) and a "complex" (22q11, 9q34 and at least one additional chromosome). Difficulties arise if a variant translocation generates "masked" Ph and also if rearrangements are suboptic. We present cytogenetic findings of 34 CML pts with vPh. Cytogenetic analyses were performed on unstimulated bone marrow cells. Standard procedures and HG-banding technique were used. Five pts (5/34) had simple vPh, while other 29 pts (29/34) had complex vPh-producing translocations. Reverse transcription-polymerase chain reaction (RT-PCR) was performed in eight pts, in order to detect the expression of BCR/ABL sequence. RT-PCR analysis showed a b3a2 fusion transcript in six (6/8) pts, and b2a2 configuration in one patient (1/8). BCR/ABL fusion was not detected in one pt (1/8) with simple vPh. Conventional cytogenetics was refined by fluorescence *in situ* hybridization (FISH) in one pt. FISH demonstrated BCR/ABL fusion gene on a short arm of chromosome 9. Variant translocations involving 9p are very rare in CML. In conclusion, applying of molecular analyses (RT-PCR or FISH) beside the conventional karyotyping is necessary in diagnosis and more precise understanding biological mechanisms occurring in vPh in CML.

**P06.172****Impact of interleukin-10, tumor necrosis factor- $\alpha$  and transforming growth factor- $\beta$  polymorphisms on the incidence and outcome of diffuse large B-cell lymphoma**

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Background. This study assessed whether interleukin-10 (IL-10) -1082A/G, -819C/T and -3575T/A, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) -308G/A and transforming growth factor- $\beta$  (TGF- $\beta$ ) c10T/C genetic polymorphisms influence the incidence and outcome of diffuse large B-cell lymphoma (DLBCL).

Patients and methods. The study included 94 patients with DLBCL and 60 ethnically matching controls with no lymphoma in family. Genotyping was performed with ARMS-PCR, RFLP and Taqman allelic discrimination assays, respectively.

Results. In DLBCL group IL-10 -819CC genotype was more ( $\chi^2$ , df=2, p=0.0035) and TNF- $\alpha$ GG less frequent ( $\chi^2$ , df=2, p=0.0052) than in controls. There was no significant difference in the incidence of relapse, presence of bulky disease and prognostic group according to International Prognostic Index (IPI) in DLBCL patients with different IL-10, TNF- $\alpha$  and TGF- $\beta$  genotypes, excluding patients with TGF- $\beta$  c10TT who were the most often scored as "low IPI" (better prognosis) ( $\chi^2$ , df=6, p=0.0146). Patients with IL-10 -1082GG (Logrank test, p=0.0307), IL-10 -3575TT (Logrank test, p=0.0404) and TNF- $\alpha$  -308GA+AA (Logrank test, p=0.0184) had longer disease free inter-

val (DFI) than patients with other IL-10 -1082, -3575 and TNF- $\alpha$  -308 genotypes. Also, patients with TNF- $\alpha$  -308GA+AA had better overall survival (OS) (Logrank test,  $p=0.0371$ ) than patients with TNF- $\alpha$  -308GG.

Conclusion. Homo- and/or heterozygous carriers of high IL-10 and TNF- $\alpha$  producer alleles were more susceptible to DLBCL (IL-10 -819CC, TNF- $\alpha$  -308GA+AA), but they had better DFI (IL-10 -1082GG, IL-10 -3575TT, TNF- $\alpha$  -308GA+AA) and OS (TNF- $\alpha$  -308GA+AA). TGF- $\beta$  c10TT carriers (low-producer genotype) had better prognosis according IPI.

#### P06.173

#### Regulation of the leukemia associated oncogene and developmental regulator EVI1 by all-trans retinoic acid (ATRA)

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The EVI1 gene codes for a zinc finger protein with important roles in embryonic development and in myeloid leukemogenesis. It is transcribed into several mRNA species with variable 5'-ends. One of these mRNA variants, MDS1/EVI1, gives rise to an EVI1 protein with an extended N-terminus and with functions partially different from those of the shorter EVI1 protein type. The other EVI1 5'-end variants are most likely translated into the same protein, i.e. the short EVI1 protein variant, but their variable 5'-UTRs can be expected to affect the regulation of protein expression. So far all-trans retinoic acid (ATRA) is the only known physiological regulator of EVI1 in mammalian cells.

Using the teratocarcinoma cell line NT-2, we have investigated the induction of EVI1 by ATRA. Time course analyses showed that ATRA rapidly induces the EVI1 mRNA in NT-2 cells. A maximum is reached after approximately 48 hrs, except for the MDS1/EVI1 mRNA, which was noticeably induced only after 48 hrs. This response was already detectable with as little as 10 nM ATRA and affected all EVI1 mRNA variants, albeit to variable degrees. Using reporter gene assays, an ATRA responsive region of ~200 bp was identified within exon 1a of EVI1. Chromatin immunoprecipitation (ChIP) experiments confirmed the binding of retinoic acid receptors RAR and RXR to a retinoic acid response element (RARE) in this region.

We conclude that the EVI1 gene is a direct target of retinoic acid signaling and that its regulation via this pathway is mediated through an intragenic RARE.

#### P06.174

#### Alterations of *herg1* isoform expressions in pediatric acute myeloid leukemia and solid tumors

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Expression of K<sup>+</sup> channels encoded by *human ether-a-go-go related gene*(*herg*) is reported to be deregulated in cancer cells. We studied the expression levels of *herg1* and *herg1b* by qRT-PCR in 35 pediatric acute myeloid leukemia(pAML) patients together with a group of human tumor samples(n=40) and their healthy counterparts. A common HERG polymorphism(K897T) was also analyzed in pAML patients. Our results suggest that *herg1* expression is lower in pAML patients compared to the control group which is composed of CD33+ cells isolated from healthy bone marrows(2.5fold). *Herg1b* expression was found to be higher in pAML patients(20 fold,  $p<0.01$ ) compared to controls. The results of *herg1* and *herg1b* expressions revealed that expression of *herg1* was found to be higher in stomach and pancreas tumors, but lower in colon and breast tumors compared to controls. Though, increased *herg1b* expression level was determined in stomach, colon and pancreas tumors while it was determined to be downregulated in breast tumors compared to controls. The presence of HERG1 and HERG1B was confirmed by western blot analysis. HERG-K897T, known to create a new phosphorylation site for Akt protein kinase, was significantly more common among pAML patients(37%). The study of the effect of K897T on PI3K signaling is ongoing. The increased level of *herg1b* expression in pAML patients and tumors must be a result

of HERG currents displaying fast deactivation kinetics that may be attributable to expression of *herg1b* in cancer cells. These suggest that HERG channel may have an oncogenic potential and *herg* may be a molecular target for both prognosis and pharmacological therapy of pAML and solid tumors.

#### P06.175

#### Outcomes of the modulation of hTERT gene expression in promyelocytic leukemia as a model

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Telomerase is a ribonucleoprotein complex. It consists of two main components, telomerase reverse transcriptase (hTERT) and telomerase RNA (hTR). High telomerase activity is present in most malignant cells, but it is barely detectable in majority of somatic cells. The direct correlation between telomerase reactivation and carcinogens has been made hTERT a key target for anti-cancer therapeutic studies. In this study, we evaluated the ability of the new generation of short interfering RNA (siRNA) to modulate telomerase activity in the human promyelocytic leukemia cell line (HL-60). Data showed that transient transfection of the cells by hTERT specific siRNA resulted in  $97.0 \pm 0.65$  down regulation of the hTERT mRNA content. Whereas hTERT protein level in treated cells were suppressed  $49 \pm 3.47$  at 24 hours post transfection. The results of Telomeric Repeat Amplification Protocol (TRAP) showed that telomerase activity was reduced  $86.6 \pm 1.99$ . Moreover, unlike most previous reports, the pattern of telomere restriction fragment (TRF) length did not show a significant difference in treated cells compared to the control samples. Furthermore inhibition of the cell proliferation rate was about  $52.8 \pm 2.3$  and the apoptotic index of leukemic cells was  $30.5 \pm 1.55$ . In summary, inhibition of hTERT exerted a direct antiapoptotic function in HL60 leukemic cells which was independent of telomere length. It promises the development of new generations of drugs for cancer therapy.

#### P06.176

#### Detection of two unusual variants of *ITPA* gene transcript in chronic myelogenous leukemia (CML) patients

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One of the most significant damages to the cells is oxidative deamination of DNA and free nucleotides in the cell pool. Incorporation of deaminated nucleotides such as ITP or dITP into DNA or RNA can increase the frequency of base substitution mutation or alter their structures. Also, it has been suggested that presence and accumulation of these rough nucleotides can lead to genetic instability which is the perquisite of different types of diseases or cancers. Inosine triphosphate pyrophosphates (ITPase) encoded by *ITPA* gene, is responsible for protecting the cells by omitting deaminated purines from the free nucleotide pool. The objective of this study was to examine the possible dysfunction of *ITPA* gene activity as an important factor in genetic background predisposing to chromosomal disorders and malignancies such as Chronic Myelogenous Leukemia (CML). We studied *ITPA* gene expression in CML patients and our results showed a significant reduction of *ITPA* gene expression in CML patients in comparison to controls. Also two unusual variants of *ITPA* transcript in addition to expected transcript were detected in some CML samples. These unusual variants were cloned and sequenced and their protein structure predicted using bioinformatics software. It revealed that these variants have nucleotide deletions in their ORF region. The results of their protein prediction showed that their proteins don't have the efficiency of catalytic activity. According to decreased expression of this gene in patients and presence of these unusual variants; it seems the function of ITPase has been altered in CML patients.

**P06.177****Somatically acquired JAK1 mutations in adult acute lymphoblastic leukemia**

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Aberrant signal transduction contributes substantially to leukemogenesis. Here, we report that somatic mutations in the Janus kinase 1 (JAK1) gene, which encodes a cytoplasmic tyrosine kinase that non-covalently associates with a variety of cytokine receptors and plays a nonredundant role in lymphoid cell precursor proliferation, survival and differentiation, occur in individuals with acute lymphoblastic leukemia (ALL). JAK1 mutations were more prevalent among adult subjects with T-cell precursor ALL, where they accounted for 18% of cases, and were associated with advanced age at diagnosis, poor response to therapy and overall prognosis. All mutations were missense, some predicted to destabilize interdomain interactions controlling the activity of the kinase. Three mutations that were studied promoted JAK1 gain of function, and conferred interleukin 3-independent growth in Ba/F3 cells and/or interleukin 9-independent resistance to dexamethasone-induced apoptosis in T cell lymphoma BW5147 cells. Such effects were associated with variably enhanced activation of multiple downstream signaling pathways. Leukemic cells with mutated JAK1 alleles shared a gene expression signature characterized by transcriptional upregulation of genes positively controlled by JAK signaling. Our findings implicate dysregulated JAK1 function in ALL, particularly of T-cell origin, and point to this kinase as a target for the development of novel anti-leukemic drugs.

**P06.178****Study of the effect of MRP1 gene polymorphisms on its mRNA expression in acute leukemic patients**

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One of the major problems in treating cancer cells is that they can acquire drug resistance (so called Multidrug Resistance: MDR). There are several mechanisms responsible for MDR. One of the most important is the overexpression of ABC transporter genes. One of the most extensively studied genes involved in MDR is multidrug resistance protein 1 (MRP1). We have shown that the overexpression of this gene is associated with the MDR in Iranian leukemic patients. However, MRP1 gene amplification could not be identified in any of those patients. Another mechanism is the influence of the MRP1 gene polymorphisms on the expression level of the gene.

We aimed to investigate the possible association between the expression level of MRP1 and occurrence of MDR in leukemic patients. Furthermore, we wished to test the hypothesis that MRP1 polymorphisms would be predictive of MDR in patients with acute leukemia. mRNA level of MRP1 was determined in 111 patients with acute leukemia (including 52 patients with AML and 59 patients with ALL) by quantitative real time RT-PCR and compared to the type of response to chemotherapy.

We typed G816A, T825C, G2168A, C2217T, G2268A, G1299T, G-260C, A-275G MRP1 polymorphisms in those patients classified either drug-resistant. We found that high expression of MRP1 was associated with MDR phenotype in both AML and ALL patients. There was no effect of a particular genotype on the expression level of the MRP1 gene. This could show the lack of dependency of any of these genotypes on the chemosensitivity in this group of patients.

**P06.179****Expression analysis of the mitogenic growth factor receptors in childhood acute myeloid leukemia; Increased Expression of Vascular Endothelial Growth Factor Receptor-1 and the Loss of Estrogen Receptor beta**

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Constitutive PI3K/Akt/mTOR signaling is upregulated by the activating mutations of receptor tyrosine kinases, autocrine/paracrine secretion of growth factors and estrogens triggering the binding of ER $\alpha$  to PI3K and ER $\beta$  to AKT. Mutational analysis of FLT3 together with the expression analysis of VEGF receptors, estrogen receptors and IGF system were performed in pediatric AML (pAML) patients and controls, CD33+ cells isolated from healthy bone marrows. FLT3/ITD and FLT3/D835 mutations have been identified in 12% and 2 % of 50 pAML patients respectively. Flt-1 and KDR expression were determined to be significantly higher in pAML patients. ER $\alpha$  expression was observed in 54.5% of the patients where diminished ER $\beta$  expression was determined. The results of the IGF system genes expression studies indicated higher IGF-1 expression (16.3 fold), and significantly lower IGF-2 and IGF-1R expressions in pAML patients. IGFBP-rP1 expression was 10.48 fold lower in pAML patients. Expression studies of initiator caspases, caspase8 and caspase9 revealed low level of caspase9 and increased level of caspase8 (13.6 fold) expression in pAML patients. IGFBP-rP1 and caspase8 expressions were also evaluated by western blot analysis in pAML samples. Upregulation of PI3K/Akt pathway through altered expression levels of upstream mitogenic growth factors and their receptors were also confirmed by altered expression levels of Akt downstream genes; c-myc and cyclin D1 as a result of GSK3 $\beta$  phosphoinhibition in pAML patients. To our knowledge this is the first data representing the loss of ER $\beta$  gene expression in pediatric AML patients. Our study showed that IGF-1 and caspase8 could be potential antiapoptotic markers and IGFBP-rP1 a new tumor suppressor in pAML.

**P06.180****Study of Suz12 gene expression in chronic myelogenous leukemia patient**

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Polycomb group proteins are transcriptional repressors that play a central role in the establishment and maintenance of gene expression patterns during development. Trimethylation of histone H3 on lysine 27, mediated by a P<sub>c</sub>G protein complex consisting of Eed, Ezh2, and Suz12, is integral in differentiation, stem cell self-renewal, and tumorigenesis. Dereregulated activity of the chromatin remodeling Polycomb Repressive Complex 2 (PRC2) has recently been shown to be a frequent event in human tumors. Recent study shows that loss of Suz12, a core component of Polycomb Repressive Complex 2 (PRC2), function enhances hematopoietic stem cell (HSC) activity. Their study suggests that PRC2 is required to maintain a specific gene expression pattern in hematopoiesis that is indispensable to normal stem cell function. Chronic myelogenous leukemia (CML) is a clonal myeloproliferative disorder of the hematopoietic stem cell (HSC).

In this study the expression level of Suz12 gene was compared between CML patients and control group. In this report we will present the obtained results.

**P06.181****Fluorescence *in situ* Hybridization Analysis of the hTERC Region in Acute Myeloid Leukemia Patients**

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Telomerase is a ribonucleoprotein complex consisting of reverse transcriptase (hTERT), proteins (hTP1) and RNA template for telomeric DNA synthesis (hTERC). The telomerase RNA component (TERC) gene is located at 3q26. Increased TERC gene dosage has been detected frequently in a variety of human cancers, suggesting a growth advantage in cells with increased gene dosage. Amplification was shown by fluorescence *in situ* hybridization (FISH) in different cancers. It has been suggested that the activation of telomerase in leukemic cells may be connected with amplification of hTERT and hTERC genes. The aim of this study was to investigate whether there

is hTERC gene amplification detectable by FISH in acute myeloid leukemia (AML) cells. The bone marrow samples of 23 newly diagnosed adult AML patients were retrospectively analyzed for this study. We did not detect a visible abnormality of the 3q region with the previous conventional cytogenetic analyses of the patients. Interphase cells were hybridized with hTERC (3q26) / 3q11 probe (Kreatech, Netherlands). We did not detect amplification of the region in patients. Although it has been reported that hTERT gene amplification may partially contribute to the increased telomerase expression and activity in leukemic cells, it is not possible to make such a conclusion with a small number of patients with the results of the current study, as we did not detect amplified hTERC in our group of patients.

#### P06.182

#### High resolution cytogenetic analysis of a clonal der(2)t(1;2)(q31.2;q24.1) in an acute myeloid leukaemia

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A 25-year old woman was admitted for an acute myeloid leukaemia, AML-M2 according to FAB classification. Bone marrow karyotype at diagnosis showed an unbalanced reciprocal translocation der(2)(t(1;2)(q31.2;q24.1)) as sole clonal abnormality, in major part of mitoses. This rearrangement was not known as recurrent, leading to a partial trisomy 1q and monosomy 2q of indefinite prognosis. After a primary resistance to induction chemotherapy, a complete remission was obtained and patient could benefit from a bone marrow transplantation. She is still in remission at 1 year post diagnosis.

Breakpoints 1q and 2q of the translocation were precisely defined by CGH array with a Perkin Elmer 5200 BAC clones chip, in order to detect a potential new fusion gene. The study performed from diagnosis bone marrow which contained 61% blast cells, confirmed the 1q and 2q imbalance, with breakpoints at 1q31.2 and 2q24.1. A FISH analysis was performed with BAC probes of the chip, located the closer from both breakpoints. 2q24.1BAC probe was deleted on der(2) but 1q31.2 BAC probe which covers 3 coding genes was entirely retained by der(2). Investigations with other probes covering the breakpoint region are in progress.

#### P06.183

#### Detection and Assessment of prognostic significance of ALK gene rearrangement in NHL patients

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The non random genetic aberrations in non Hodgkin lymphoma (NHL) involving anaplastic lymphoma kinase (ALK) gene, t(2;5)(p23;q35) creates NPM-ALK fusion gene, with tyrosine kinase activity responsible for its oncogenic property; by activation of downstream effectors such as phospholipase C. Thus this work aimed to detect ALK gene (2p23) rearrangement in various types of NHL by conventional cytogenetics (CC) and fluorescence in situ hybridization techniques (FISH), and to compare between the results of both techniques. To delineate this aim, ALK gene rearrangement was investigated in 25 newly diagnosed grade IV adult NHL patients. FISH analysis revealed positive ALK rearrangement in 5 (20%) cases, 4 of them were diagnosed as anaplastic lymphoma and one as DLBCL in comparison to absolute negative detection of t(2;5)(p23;q35) by CC; proving the superiority of FISH in detection of specific genetic aberrations. According to the results of FISH analysis, patients were divided into: Group I: with normal ALK signals, Group II with ALK aberrations.

In conclusion, ALK gene rearrangement was detected in NHL patients with different histopathological subtypes, showing maximum expression in ALCL. It was correlated with good patient outcome, indicating its importance as an important prognostic factor and its potential as a promising target for therapeutic intervention. FISH technique possessed the upper hand in detection of ALK rearrangements as an example of specific targeted aberrations in comparison to CCA. Nevertheless, conventional karyotyping remained the cornerstone in cytogenetic analysis because of its ability to simultaneously scan for various aberrations affecting many chromosomal loci.

#### P06.184

#### Chromosomal abnormalities in 50 patients with B-cell chronic lymphocytic leukemia detected by array comparative genomic hybridization

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B-cell chronic lymphocytic leukemia (B-CLL) is the most common adult leukemia. The progress in molecular genetic characterization of B-CLL confirmed the prognostic role of IgV<sub>H</sub> mutational status, as well as chromosomal abnormalities defined by molecular cytogenetic methods. However, besides chromosomal changes with known prognostic impact, such as deletions of 6q, 11q (ATM), 13q, 17p (TP53) detected routinely, the other additional abnormalities can be found. It is presently not clear whether these aberrations have any impact on prognosis and disease progression. To detect abnormalities escaping from routine FISH investigation, we performed array comparative genomic hybridization (arrayCGH) of 50 B-CLL patients diagnosed recently in our center. The aim of this study was supported by the fact, that gains and losses of genetic material can lead either to oncogenic activation of protooncogenes or to loss of tumor-suppressor genes function, within the gained and deleted regions respectively. ArrayCGH revealed copy number changes in 43 out of 50 patients. Except already well known changes as 6q- (7 pts.), 11q- (13 pts.), 13q- (28 pts.), 17p- (5 pts.) and +12 (5 pts.), we detected also other recurrent abnormalities as 2p+ (11 pts.), 8q+ (4 pts.), 14q- (3 pts.), abnormalities of chromosome 18 (9 pts.) and others. Cases with detected 6q- were subjected to 32K tiling path chromosome 6 arrayCGH in order to define the minimal commonly deleted region more precisely. We will present a summary of cytogenetic, FISH and arrayCGH findings in 50 B-CLL patients.

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#### P06.185

#### Chromosomal abnormalities in patients with B-cell chronic lymphocytic leukaemia from a single center

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B-Cell chronic lymphocytic leukemia (B-CLL) is the most frequent leukaemia in the Western world. This pathology is characterized by the clonal expansion of morphologically mature small lymphocytes. Conventional cytogenetics shows chromosomal abnormalities in 40-50% of the cases, whereas FISH can detect about 80% of abnormalities. The most common chromosomal abnormalities by FISH are del 13q14.3 in 50% of the cases, 20% trisomy 12 and less frequently deletions of 11q22-23, 17p13 and 6q21. These alterations are associated with clinical course: 17p13, 11q22-23 and del6q are linked with a shorter survival, followed by trisomy 12 and the normal karyotype and del 13q14.3 is associated with favorable clinical course.

From a single center, 171 samples with B-CLL have been analyzed using conventional and molecular cytogenetics. Successful results have been achieved in 90% of the cases by conventional cytogenetics and 94% by FISH. The rate of abnormality detections by conventional cytogenetics was 53% (81/154) and 80% (129/161) by FISH. These rates are in accordance with WHO. The results by molecular cytogenetics were: 58% of the cases showed del 13q14.3, 22% had deletions of 11q22-23 and trisomy 12 and deletion of 17p13 had minor frequency, 17% and 14% respectively. However, 28% (43/154) of the cases had alterations which had not been detected by FISH.

The study of CLL by conventional cytogenetics in all the samples will be discussed to detect more abnormalities. This information will be useful for the study of other mechanisms implicated in this pathology and for the obtention of information for prognosis.

#### P06.186

#### Cytogenetic studies among Iranian patients with blood cancers

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Cytogenetic studies are important diagnostic and prognostic factors in evaluating patients with blood cancers. More than half of all leukemias

mias have detectable chromosomal aberrations by karyotypic analysis. Many chromosomal abnormalities associated with particular subtypes of leukemia, and appear to have prognostic significance. It has been suggested that some chromosomal abnormalities, such as t(1;3), t(1;7), t(1;19), t(2;8), t(2;11), t(8;14), t(8;21), t(8;22), t(9;11), t(9;22), t(11;14), t(15;17), del(5), del(7), del(11), del(13) are associated with particular subtypes of leukemia, and some of them are poor prognostic indicators. Today cytogenetic studies are an important tools which can help Clinical Oncologists to predict a good or poor response to treatment in patients with different types of leukemia. We are presenting chromosomal studies among 79 patients which referred to our cytogenetic laboratory from Hematologists/Oncologists with the preliminarily diagnosis of blood malignancies, during the period of one year. The table below shows the number and percentage of abnormal karyotypes among our patients.

Disease	Number of Normal Karyotype	Per cent	Number of Abnormal Karyotype	Per cent	TOTAL
Acute Lymphoblastic Leukemia	4	50%	4	50%	8
Acute Myeloid Leukemia	1	16.7%	5	83.3%	6
Chronic Lymphoblastic Leukemia	5	45.5%	6	54.5%	11
Chronic Myeloid Leukemia	5	27.8%	13	72.2%	18
Myelodysplastic Syndrome	11	100%	0	0%	11
Lymphoma	0	0%	1	100%	1
Multiple Myeloma	0	0%	3	100%	3
Thrombocytopenia & Leukopenia	2	100%	0	0%	2
Severe Anemia	3	75%	1	25%	4
Unrecognized	9	60%	6	40%	15
<b>TOTAL</b>	<b>40</b>	<b>50.6%</b>	<b>39</b>	<b>49.4%</b>	<b>79</b>

#### P06.187

#### Fluorescence *in situ* Hybridization Results of Chronic Lymphocytic Leukemia Patients

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Cytogenetic abnormalities are found in 40-50% of chronic lymphocytic leukemia (CLL) patients. Conventional cytogenetics is a basic method for detecting various abnormalities in CLL. However, conventional cytogenetic analysis is sometimes not successful or does not yield abnormal metaphases because of the low mitotic index of the malignant cells. Fluorescent *in situ* hybridization (FISH) analysis is frequently used to increase the detectability of chromosomal aberrations, since nondividing interphase cells can be analyzed in a rapid, sensitive and accurate way by this method. We evaluated the common recurrent chromosomal aberrations in 68 CLL patients by FISH analysis in different combinations of the probes 11q22.3 (ATM) (n= 62), 13q14.3 (13S25) (n= 58), CEP12 (n=22) and 17p13.1 (TP53) (n=42). Of the 68 patients analyzed by FISH, 35 (51.5 %) had at least one aberration. Five patients (7.35%) had two abnormalities and one patient (1.5%) had three abnormalities. The most frequent aberration was 13q14 deletion (45.8%), followed by trisomy 12 (27.3%). In 12 patients, all four probes were analyzed and 10 (83.3%) patients were found to have at least one abnormality; suggesting that analyzing all four probes might be a better approach in evaluating CLL patients. Chronic lymphocytic leukemia (CLL) has a highly variable clinical course and the genetic abnormalities underlying this heterogeneity are frequently being investigated. Along with the clinical markers, molecular cytogenetics is a valuable tool to help predict the individual prognosis of the patients.

#### P06.188

#### The importance of complex chromosomal rearrangements in evolution of patients with chronic myeloid leukemia

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Background: Chronic myeloid leukemia (CML) is one of the most malignant diseases studied because of the chromosomal aberration in bone marrow cells of patients in chronic phase of CML. During pro-

gression of the disease from the chronic to the accelerated phase (AP) and/or blast crisis (BC), clonal evolution with secondary numerical and structural aberrations is frequently observed. These secondary chromosomal aberrations are demonstrable in 80% of cases and consist in some abnormalities being +19, +21, +7, +8, +Ph.

The aim of our study was to investigate complex chromosomal rearrangements found in the bone marrow cells of 25 patients with CML by cytogenetic and molecular methods; to determine the chromosomal parts which are involved in complex chromosomal rearrangements during progression of the disease.

Methods: Among 52 patients studied 25 patients were with CML and complex chromosomal rearrangements (15 males and 10 females). The study took place between 2002-2008. Chromosomal preparations were made by standard techniques and G-banding with Wright's stain. We defined as a complex karyotype more than two chromosomal abnormalities and/or more than three breakpoints. In some patients we performed further molecular analyses using real-time RT-PCR.

Results: Variant Ph translocations were found in 12 patients, the rest of them had a classical Ph translocation associated with additional structural aberrations. The most frequent chromosomes involved into complex chromosomal rearrangements were found 11(x3), 17(x6) and 19(x3).

Conclusions: Complex chromosomal rearrangements are associated with poor prognosis and the genetic mechanisms are playing a role in the progression to the blastic phase of CML.

#### P06.189

#### Emergence of clonal chromosomal abnormalities in Philadelphia negative cells of chronic myeloid leukemia following successful treatment with Imatinib

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Imatinib is a tyrosine kinase-specific inhibitor used for the treatment of chronic myeloid leukemia(CML),it produces sustained complete hematologic and cytogenetic responseCR in CML patients. Studies reported the occurrence of additional cytogenetic abnormalities in Philadelphia chromosome(Ph)-negative cells emerging after suppression of Ph-positive clone. These abnormalities were described in a relatively high proportion of patients treated with Imatinib, however, the origin of these abnormalities as well as their biological and clinical significance remain to be clarified. We investigated cytogenetic follow-up features of chronic myeloid leukemia patients who developed abnormalities in Ph-negative cells during imatinib treatment.

In a cohort of 251patients with Ph-positive CML treated with Imatinib,5%(n=11) developed clonal chromosomal abnormalities in Ph-negative cells. The median interval of the first anomaly observation was 15months(range:9-36months), thus, 5patients demonstrated complete CR, 4patients had major CR, and 2patients had minimal CR. The most common cytogenetic abnormality was trisomy8, documented in 8patients, monosomy7 was observed in one patient, nullisomyY in one patient and unusual translocation t(7;12)(q11;p13) was identified in one patient. After a median follow-up of 28months, cytogenetic evaluation of 6data available, revealed 4patients in complete or major CR, 1patient in minor CR and 1patient in minimal CR.

Our findings raise the questions of potential adverse cytogenetic events in CML patients with good responses to Imatinib; and also, about potential mechanisms underlying the development of secondary clonal cytogenetic changes in Ph-negative cells of Imatinib treated patients. Whereas, the answers to these questions are premature and will require further study, including additional clinical and cytogenetic follow-up

#### P06.190

#### Research on chromosomal (Chromosomes No. 1-9) aberrations in chronic myelocytic leukemia by MLPA method

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In this study, it is aimed at investigating chromosomal aberrations in Chronic Myeloid Leukemia (CML) and particularly the aberrations

on chromosome regions of nearly 33 genes related with oncogenes (MYB, MYC, NRAS and etc.), transcription factor (RENT2, NFKBIA and etc.), signal transmission (PIK3CA, IMPDH1, PTPRD and etc.), cytokines (IL1, TANK, IL13 and etc.), immune system (TGFBR1, PT-P4A3) and apoptosis (MOAP1, BAX, PDCD8 and etc.) by method of MLPA (Multiplex Ligation-dependent Probe Amplification). BCR-ABL positive (p210 chimeric protein) 48 patients and 15 healthy individuals were included in the study. The aberrations on different regions of 9 chromosomes, which carried 33 genes, were investigated. Results were analyzed by comparing both among each other and with clinical parameters. As a result of MLPA analyses, duplication was detected in fibroblast growth factor receptor 1 (FGFR1) gene in 2 CML patients, in inosine 5' monophosphate dehydrogenase 1 (IMPDH1) gene in 4 CML patients and in Postmeiotic Segregation Increased, S. Cerevisiae, 2 (PMS2) gene in 1 CML patient. As a result of comparison with clinical parameters, it was understood that presence of duplications reduce percentage of survival without progression for 7 years and a significant relationship is found between duplication and the response to imatinib therapy in our patient group. In this study, the duplications of 3 genes in chromosome 7 and 8 were detected for the first time in CML and it was shown that chromosomes 7 and 8 should be the center of more detailed studies.

#### P06.191

##### Cytogenetical follow-up of patients with chronic myeloid leukemia Ph1+, treated with Gleevec - the second report

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GleevecR (imatinib mesylate) is a potent inhibitor of the protein tyrosine-kinase formed under the translocation t(9;22), characteristically for chronic myeloid leukemia (CML). In our center, the treatment with GleevecR received for CML patients has been initiated since the year 2002. This is the second report about the evolution of cases (2004 the first report). 27 patients were included in this study: 15 men and 12 women. The average age was: 42.30 years (women), 36.66 years (men). The medium duration of the treatment was 20.8 months. We have performed a cytogenetic study in 24-48h nonstimulated cultures of bone marrow cells from all patients with CML at diagnosis and during the treatment. The GTG analysis revealed the karyotypes. Cytogenetical evaluation was made 6 months later, during the first year and then every 12 months. We have considered a complete cytogenetical response (CCR) if there are 0% Ph1+ metaphases, a major cytogenetical response (MCR) if there are less than 35% Ph1+, a minor cytogenetical response (mCR) if between 35-95% Ph1+ and absent cytogenetical response if there is 100% Ph1+. At 12 months 75% obtained CCR, 16.66% MCR and 8.33% mCR. At 24 months, 76.92% obtained CCR, 15.38% MCR and 7.69% mCR. At 36 months, 80% obtained RCC and 20% mCR. At the last evaluations, 67.85% obtained CCR, 10.71% MCR and 21.42% mCR. Clinical-cytogenetical correlations will be presented in extent. The results confirm the efficiency of Imatinib in controlling Ph1+ clone and prove that Imatinib is a first line treatment option for CML.

#### P06.192

##### Treatment with Imatinib in chronic myeloid leukemia. The role of Philadelphia chromosome in monitoring of the therapy

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Background: Chronic myeloid leukemia (CML) has substantially improved survival with the application of Imatinib, however, the patients with advanced CML such as in the accelerated phase (AP), or black crisis (BC) have bad prognosis even in the era of tyrosine kinase inhibitors. Aims: To analyse the hematological and cytogenetic response to Imatinib of chronic phase Ph 1+ CML patients and the importance of cytogenetic monitoring of those patients. Methods: We analysed 58 CML patients diagnosed and treated in the Hematology Department of Timisoara, between 2002 - 2007. Were included in this study 48 patients in chronic phase and 10 patients in advanced phase of the disease. Response criteria was NCCI evaluated. Patients received 400 mg Imatinib every day. Standard cytogenetics was performed at diagnosis, at 6, 12 and 24 month. Results: Survival at 5 years was: 10 pa-

tients died with blastic phase, 7 patients evolved to accelerated phase and received Imatinib 600 or 800 mg. There are still alive 48 patients, 42 in chronic phase. Complete cytogenetic response (CCR) was seen in 34 patients. This patients are monitored at 6 month with standard cytogenetics and molecular analysis by quantitative PCR (RQ-PCR, TagMan). 16 patients lost CCR, in 10 patients accelerated or blastic phase was seen in bone marrow and blood. Conclusion: Conventional cytogenetic monitoring is an important evaluation method of the Imatinib response in CML. It is necessary to complete the evaluation with molecular analysis that could announce the disease progression earlier than cytogenetics.

#### P06.193

##### Prognostic value of chromosomal aberrations in relapsed multiple myeloma patients treated by thalidomide

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We have focused on four structural and one numerical chromosomal aberration: del(13)(q14) (RB1 gene), del(17)(q13) (p53 gene), t(4;14) (IGH/FGFR3 genes), gain/amplification (1q21) (CKS1B gene) and non-hyperdiploidy. All of these aberrations are known as negative prognostic factors in patients treated by conventional therapy or stem cell transplantation.

The aim of this study is to determine the prognostic value of these selected aberrations in relapsed patients treated by novel agent thalidomide.

We have an increasing group of (recently 43) MM patients. Their characteristics at the start of treatment: Average age 68 years, 76 % (35/43) were in the clinical stage III. and 84,7 % (39/43) in stage A. 58,1 % (25/43) patients reached overall response during the follow up (median 15.6 months).

For identification of plasma cells in bone marrow samples we have used the AMCA antibody based immunofluorescent labeling protocol or MACS technique. For detection of chromosomal abnormalities the I-FISH technique has been used.

Cytogenetic findings: del(13)(q14) was found in 42 % (16/38) patients, del(17)(q13) in 8 % (3/37), translocation t(4;14) in 20 % (7/35), amplification of 1q21 in 47,2 % (17/36) and non-hyperdiploidy in 60 % (18/30) patients.

Our results suggest that no one of monitored aberrations seems to have any impact on efficiency of used treatment. It is possible that thalidomide overcome the negative impact of cytogenetic aberrations except gain/amplification 1q21. We will continue in this research to reach larger data set to confirm or disconfirm our results.

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#### P06.194

##### MLL amplification in patients with acute myeloid leukemia (AML)

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Protooncogene activation may be caused by mutation, DNA rearrangements or gene amplification, subsequently leading to cell cycle disruption and tumorigenesis. Gene amplifications are produced by various mechanisms which often results in overexpression of oncogenic protein. Recent reports have identified transcriptional regulatory factor MLL gene amplification as a potential mechanism of leukemogenesis in hematological malignancies.

The aim of this study was to assess frequency and mechanisms of MLL amplification in de novo AML and its correlation with clinical features. During years 2006-2008, bone marrow samples of 86 adult patients were examined using conventional cytogenetic analysis and FISH with LSI MLL Dual Color Break Apart Rearrangement Probe (Abbott

- Molecular). mFISH/mBAND 11 with the probe kits from MetaSystems were used to identify the breakpoints and complex chromosomal rearrangements. The MLL gene amplification/duplication was detected in 6 patients (7%). Of these, 11q partial trisomy was found in two and multiple amplification of MLL gene within complex karyotype in two patients, MLL 5' end multiple amplification with subsequent insertion into short arm of chromosome 10 in one patient. Chromosome 11 trisomy was proved in one patient. The prognosis of patients with MLL amplification is poor. In our cohort 4 patient died, one is alive seven months after bone marrow transplantation, another one two months after diagnosis. Molecular cytogenetic and clinical data will be presented in detail.

Conclusion: MLL amplification/duplication are differentially manifested and molecular analyses are needed to clarify all cryptic aberrations undetectable by conventional techniques.

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#### P06.195

##### Case report of rare MLL-AF1q fusion resulting from t(1;11)(q21;q23) in childhood acute myelomonocytic leukaemia

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A 13 month-old girl was admitted to our Unit due to fever (39,5°C). On physical examination, she was in good condition, with pallor. Liver was palpable 1cm bellow the costal margin. Facial petechial exanthema was noticed.

Laboratory work-up showed: Peripheral blood tests: Hb 5.9gr/dl, Ht 18.3%, WBCs 56900/mm<sup>3</sup>, blasts 48%, PLTs 27000/mm<sup>3</sup>. The bone marrow aspiration revealed infiltration with blasts 60%. The cerebrospinal fluid was normal. Chest X-ray, ultrasound and CT of the abdomen were normal. Serological tests for HSV 1 and 2 were negative, EBV IgG positive, IgM negative and CMV IgG positive, IgM negative. Immunophenotypic analysis of blasts revealed Acute Myelomonocytic Leukaemia (M5b) CD33+, CD64+, CD15+, CD11b+, CD 4+, cMPO+, CD56+/-, CD13 +/-.

Conventional and molecular cytogenetics analysis of BM were performed. FISH analysis using LSI MLL t(11q23), EVI,inv(3)(q26),t(3;3) and LSI PML/RAR $\alpha$ (15;17)(q22;q21.1) revealed rearrangement only for the MLL gene in 90.9% of the nuclei that were analyzed. Conventional cytogenetic analysis revealed a clone of t(1;11)(q21;q23) on both, bone marrow and peripheral blood samples.

She received chemotherapy according to AML-BFM-2004 protocol. She received cytoreductive prephase with 6-thioguanine and cytarabine because of high leukocyte count. Reduction of WBC was observed on the same day.

On day 15 in bone marrow, morphologically, blasts were <10% and FISH studies showed 38.5 % MLL positive. MRD was not detected by flow-cytometry.

On day 21 bone marrow was morphologically in remission but FISH analysis detected 5.7% MLL positive.

She continued chemotherapy. On day 49, the bone marrow was in morphological and molecular cytogenetics remission.

#### P06.196

##### Importance of Culture Medium in Multiple Myeloma

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Multiple myeloma (MM) is a disease originating from plasma cells with low proliferative index. Plasma cells begin DNA synthesis and cell division in only advanced stages of the disease. MM is heterogeneous in its clinical and genetic properties and cytogenetic studies are valuable diagnostic tools in the disease diagnosis and follow up. Existence or absence of chromosome abnormalities is important in predicting the answer to therapy regimens, event free and overall survival of the patients. Conventional cytogenetics has been the basic method to evaluate karyotype results of the patients. Thus, culture methods to obtain chromosomes are important. In the current study, we compared our culture results in 222 MM patients whose bone marrow or peripheral blood samples were studied between January 2002 and 2009. We di-

vided the patients in two groups according to the culture media used during the analyses. There were 15 patients for whom uninduced lymphocyte cultures made in RPMI 1640 containing 10% fetal bovine serum and antibiotics was used and 207 patients for whom bone marrow specific commercially available culture media were used. We observed that culture success rates increased from 66.67% in the first group to 71.98% in the second group. Also, chromosome quality improved in the second group enabling detailed chromosome analyses, thus abnormality detection rates increased to 11.59% from null in the second group, emphasizing the importance of specific culture media.

#### P06.197

##### Clinical and prognostic implication of cytogenetics in the multiple myeloma patients

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**BACKGROUND:** Multiple myeloma (MM) is a malignant neoplasm of plasma cells that accumulate in the bone marrow. As in other hematological malignancies, cytogenetics is becoming a major prognostic parameter in myeloma. Fluorescence in situ hybridization (FISH) is more sensitive than conventional cytogenetics for recognizing chromosomal changes. **OBJECTIVE:** To investigate the clinical significance and the prognostic role of 17p13 deletions and t(4;14)(p16;q32) in MM patients. **METHODS:** We analyzed the prognostic value and the clinical implication of FISH testing in 21 MM patients treated in our clinic between January 2006 - May 2008. Patients were tested to detect 17p13 deletions and t(4;14). **RESULTS:** The positive rates of 17p13 del and t(4;14) were 43% (9 patients) and 38% (8 patients) respectively. Four patients (19%) had both two abnormalities and 13 patients (62%) had at least one abnormality. Time to progression and overall survival was significantly shorter for patients with both t(4;14) and 17p13 del, than those with del 13 alone (16.6 vs 23.5 month). The risk ratio for t(4;14) was greater than for del 13 (2.4 vs 1.4) in a multivariable analysis and they are relatively independent negative factors for response to therapy. **CONCLUSIONS:** Interphase FISH is a sensitive method to investigate the cytogenetics of MM. Del 17p13 and t(4;14)(p16;q32) can be used to predict treatment response and prognosis. Identifying high-risk patients for more aggressive therapy is a critical step for improving their clinical course. It is of interest how could we integrate these cytogenetic abnormalities in the International Staging System for MM.

#### P06.198

##### Amplification 12q12-q15 is associated with adverse clinical outcome in Non-Hodgkin Lymphoma

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Amplification 12q12-q15 is one of the most frequently amplified chromosomal regions in NHL. We have undertaken a comprehensive analysis of the chromosomal structure of 12q12-q15 in FL and DLBCL, using molecular cytogenetic techniques to define the boundaries of the amplicon, delineate the minimal core domain, and determine the genes contained within this segment for correlation with existing expression profile data and clinical outcome. Forty-three NHL cases demonstrating 12q+ abnormalities by G-banding were further verified by MFISH analysis. Multicolour band analysis for chromosome 12 was used to refine the amplified region to 12q13.1-q14.3, which revealed a variety of configurations (tandem duplications, ring chromosomes) and variable copy numbers (2-8 times). Locus-specific FISH was performed using 17 BAC probes for 12q12-q15 prepared from an RPCI-11 BAC contig. Five different patterns of duplication or amplification of this region were identified, ranging from whole chromosome trisomy, whole arm duplication, to regional duplication of variable size. The borders of this amplicon extended across the q-arm from centromeric bp 53889578 to telomeric bp 71883142, corresponding to chromosomal bands 12q13.1-q14.3. This region of amplification spans ~17.3 Kb, which contains the genes ATF7, CDK2, CDK4, ERBB3, RAP1B, GLI, IFNG, MDM2, SAS and RAB21. The core amplicon, however, was only 11.6 Kb long with amplification of SAS, CDK4, RAP1B and MDM2 and was involved in all analyzed cases. These results suggest that candidate genes within 12q13-q14.3, such as SAS and MDM2, are

preferentially duplicated or amplified leading to up-regulated expression, contribute to tumour progression, and are associated with adverse outcome.

#### P06.199

#### A novel chromosomal translocation t(11;14)(q24.1;q32) involving IGH in childhood B-cell precursor acute lymphoblastic leukaemia (BCP-ALL)

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Rearrangements involving IGH gene on chromosome 14q32.3 are well known in mature B-cell malignancies and have been more recently described in BCP-ALL. IGH translocations are usually reciprocal and bring genes on other chromosomes into close apposition with the IGH locus, where their expression is deregulated due to the presence of potent B-cell-specific transcriptional enhancers. A two-year-old girl was diagnosed with an ALL common type and treated according to AIEOP-ALL-00 Protocol. Death occurred after four months due to haematological toxicity. Cytogenetic analysis of PB and BM blasts revealed a t(11;14)(q24.1;q32). FISH analysis with IGH break-apart probe confirmed the rearrangement of the IGH locus between chromosomes 11 and 14. Cloning by LDI-PCR localized the breakpoint on chromosome 11q24.1 within the intronic region 1 of BC089451, a non coding gene. Quantitative real-time PCR showed over-expression of BLID mRNA, located 14Kb downstream the BC089451 gene. BLID codes for a protein containing a BH3-like domain essential for apoptosis. FISH studies performed with 11 close BACs to confirm the breakpoint junction identified a 585Kb deletion on der(11), with complete SORL1 loss. The functional consequence of BLID over-expression due to IGH enhancer juxtaposition is currently unknown. Mutational analysis of BLID BH-3 like domain is ongoing. The translocation to the Ig locus may result not only in deregulated expression of the incoming oncogene, but also in mutations due to the action of the Ig somatic hypermutation mechanism. In our case, a mutation of BLID in BH3 domain could result in a protein not inducing apoptosis.

#### P06.200

#### Four new chromosomal translocation in B-cell chronic lymphocytic leukemia

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B-cell chronic lymphocytic leukemia (B-CLL) is the most common type of leukemia in the Western countries and is characterized by the clonal expansion of morphologically mature small lymphocytes. Clonal chromosome abnormalities in B-CLL are detected in 40-50% of cases by conventional cytogenetics after mitogen stimulation.

We report four novel chromosomal translocations identified by conventional cytogenetic in four patients with B-CLL.

All patients were male. Clinical, laboratory and immunophenotypic data were consistent with B-CLL diagnosis. Cytogenetic studies were carried out in peripheral blood (PB) using 12-O-tetradecanoylphorbol-13-acetate (TPA) as mitogen. Cultures were maintained for 3 days in CO<sub>2</sub> atmosphere.

The karyotypes were:

Case 1:

46,XY,t(1;7)(q34;q11)[2]/46,XY[18].

Case 2:

45,XY,der(7,14,15)t(7;14)(p10;q32)t(7;15)(p10;q10),del(11)(q22q23)[2]/44,idem,der(13;14)(q10;q10)[6]/46,XY[11].

Case 3:

46,XY,-8,der(17)t(8;17)(q10;p13)[8]/46,XY[6].

Case 4:

46,XY,t(15;22)(q26.1;q11.2)[13]/46,XY[7]

A search in the National Cancer Institute Mitelman Database of Chromosome Aberrations in Cancer revealed that none of the translocations reported herein has previously been described in B-CLL.

Cytogenetic analysis of B-CLL patients, despite inherent technical limitations, has provided important information on disease biology and clinical outcome. Thus, B-CLL associated chromosomal aberrations will be identified at increased frequencies, possibly enabling more precise definition of cytogenetic risk subgroups and providing new prognostic markers.

#### P06.201

#### New variant of BCR/ABL translocation, t(4;9;22) identified at the onset of chronic myeloid leukemia - case report

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Variant forms of Philadelphia (Ph) positive patients with chronic myeloid leukemia (CML) characterize ~ 5-12% of cases. The variant translocations involve one or more chromosomal regions in addition to 9q34 and 22q11.

Here we present a variant translocation, t(4;9;22) found in a patient with chronic myeloid leukemia, at the time of initial diagnosis.

Classical cytogenetics and fluorescence *in situ* hybridization (FISH) with WCP 9(R), WCP 4(G), WCP 22(R), BCR/ABL double fusion (Poseidon-Kreateck) and BAC/cosmid n85a3 (22q13.3), RP11-492I23 (4p16.3) probes were used to study the mechanism of variant Ph translocation.

Philadelphia chromosome was visible in all bone marrow cells by conventional cytogenetic analysis and karyotypes showed two other derivative chromosomes which could not be elucidated by GTG banding. By RT-PCR, a b3a2 transcript was revealed, with the cDNA amplicon shorter (between 300 and 350bp) than positive control (388bp), possible the breakpoint is into b3 exon of BCR gene.

FISH analysis allowed us to establish the following karyotype formula: 46,XY,t(4;9;22)(q21;q34;q11). ish t(4;9;22) (wcp4+,wcp22+,BCR+, RP11 -492 I 23 +, n 85a3 ;wcp9+,wcp4+,ABL+,BCR-, wcp22+, BCR+,ABL+).

Our results underline the necessity to accomplish the karyotype investigation by molecular and FISH techniques to prevent the erroneous reporting and inappropriate disease management.

#### Acknowledgments

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#### P06.202

#### Putative association of polymorphisms in DNA repair genes with cytogenetic subgroups in B-cell chronic lymphocytic leukaemia

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Genetic polymorphisms in DNA repair genes may influence the susceptibility to different forms of cancer. Therefore we investigated the association of seven SNPs in five DNA repair genes with the incidence of chronic lymphocytic leukaemia (CLL). We analysed 461 CLL patients and an equal number of sex and age matched controls using PCR followed by digestion with restriction enzymes. The odds ratios (OR) and P-values were calculated by logistic regression analysis. As chromosomal aberrations are important prognostic markers in CLL, we paid particular attention to 133 patients with the favourable cytogenetic aberration del(13q) as sole aberration and 69 patients with the unfavourable cytogenetic aberrations del(17p) and del(11q). The rare genotypes of rs13181 in the nucleotide excision repair gene *xeroderma pigmentosum D* (XPD) and rs25487 in the base excision repair gene *X-ray repair complementing defective repair in Chinese hamster cells 1* (XRCC1) occurred significantly more frequently in patients with unfavourable cytogenetic aberrations compared to controls: The genotypes of rs13181 were differently distributed under the co-dominant model (A/A vs. G/G: OR = 2.66, p = 0.024) and those of rs25487 under the dominant model of inheritance (A/C and C/C vs. A/A: OR = 2.44, p = 0.01). Additionally, significant differences in the genotype distribution of rs13181 were observed between all patients and controls (A/C and C/C vs. A/A: OR = 1.37, p = 0.03). Our results indicate that inborn polymorphisms in DNA repair genes may help to predict the outcome of CLL. We are verifying them now by correlation with survival data.

**P06.203****Molecular characterization of Turkish patients with familial hemophagocytic lymphohistiocytosis**

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The aim of this study was to elucidate molecular basis of familial hemophagocytic lymphohistiocytosis (HLH) which has become an important health problem in Turkey. Possibly familial HLH 143 Turkish patients with either family history, consanguinity and/or relapses were studied for 3 genes and chromosomal locus 9q21.3-q22 described to date. Haplotype analysis for Perforin gene revealed homozygosity in 36 families. Sequencing showed homozygous 5 different mutations in 15 of the families. W374X detected in 9 of the families was first common mutation in Turkish population. The mutations G149S was detected in 2, A91V in 2, V50M in 1, novel A593D in 1 patient and an asymptomatic sibling who developed the disease afterwards. In Munc13-4 gene, homozygosity/consanguineous common alleles were detected in 29 families. Sequencing of the gene revealed 6 different mutations in 11 of the families. R214X, identified in 7 patients from 6 of the families, was second common mutation. Homozygous R1065X, 627delT, L1044R, novel 2135-2137delTCG and R414C were observed in the rest 5 families. In Syntaxin 11 gene, homozygosity/consanguineous common allele were shown in 26 families. Third common homozygous mutation, c369-370delAG/c374-376delCGC, was identified in 7 unrelated families from distant cities. Haplotype analysis revealed that this mutation emerged from a common ancestor (founder effect). First prenatal diagnosis was given in the gene. In addition, a novel homozygous E206K mutation was identified in 2 patients of another family. No homozygosity was detected for the chromosome 9 genomic locus. This study was supported by TUBITAK (Project No: 105S396-SBAG 3193).

**P06.204****Primary myelodysplastic syndromes (MDS)- prognostic impact of additional chromosomal aberrations to 5q-**

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**Background:** Deletion of the long arm of chromosome 5 is the most frequent chromosomal abnormality in MDS (10-15% of MDS cases). Patients with del 5q, particularly those with the "5q-syndrome" have a much better prognosis than other MDS subtypes. Presence of abnormalities additional to del 5q has been suggested to negatively influence this favorable outcome. **Aim:** To analyse the prognostic value of cytogenetics aberrations additional to del (q5) in a cohort of patients with MDS. **Methods:** We studied 124 MDS patients diagnosed and treated in the Hematology Department of Timisoara between January 2001 - January 2007. Diagnosis was made according to FAB Group criteria. FAB subtypes were: 52,1% RA, 12,6% RARS, 17,65% RABE, 13,45% RABET+, 4,2% CMML. Cytogenetic analysis showed 27 patients with del (q5). From those, 12 had del (q5) associated with 1 additional anomaly, 3 patients had 2 anomalies, 4 patients had 3 anomalies and 5 patients had 4 anomalies. Additional anomalies observed in those patients were: 7 monosomy, 7q, 8, 11 and 13 trisomy. Medium survival in this group was 41 month, survival of the patients with isolated 5q deletion and only one additional anomaly was 62 month and for the patients with 2, 3 and 4 anomalies was 50, 30 and respectively 8 month. **Conclusion:** Patients with 5q deletion in association with 2 or more additional chromosomal anomalies have negative prognostic in global survival compared to those with only one or no additional anomaly to 5q deletion.

**P06.205****KRAS gene mutation testing - an inter-laboratory validated workflow for quality - assured, clinical-grade results using capillary electrophoresis**

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In recent years, the research and clinical management of cancer have changed and been revised on the basis of genetic features that characterize the specific malignant neoplasm. The KRAS gene is an oncogene that has established to play a fundamental role in the colorectal cancer therapy. It has been widely attested that novel therapeutic agents, based on monoclonal antibody targeting the epidermal growth factor receptor (EGFR), are effective only in a subset of patients. Activating mutations in the KRAS gene are found in 30-40% of colorectal tumours and are associated with poor response to anti-EGFR therapies. Thus, the KRAS mutation status can predict which patient may or may not benefit from anti-EGFR therapy. For this reason in 2007 the European Medicines Agency granted a conditional marketing authorization for EGFR therapeutic agents intended for the treatment of metastatic colon carcinoma with non-mutated KRAS (Doc.Ref. EMEA/405113/2007). This decision led to the requirement of a KRAS mutation test in the clinical practice and underlined the need for an easy-to-operate test that generates quality-assured, clinical-grade results. In this work we describe an inter-laboratory validation involving two laboratories and Applied Biosystems for a KRAS gene sequencing protocol. We developed an improved workflow from DNA to data analysis on the latest generation of capillary electrophoresis instruments with highest level of data quality and accuracy. The protocol has been performed on 50 paraffin embedded colon rectal cancer samples.

**P06.206****KRAS mutations in human endometrium. A report on 106 cases.**

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Endometrial cancer (EC) is one of the most common gynecologic malignancies in the industrialized world. The majority of cases fall in the subgroup having a favorable prognosis, the type I EC. Activating somatic mutations in the KRAS gene are common in both type I EC and its precursor lesion - the endometrial hyperplasia (EH). However, their relation to patient clinicopathological characteristics have remained largely unclear.

We have sequenced exon 1 (containing codons 12 and 13) of the KRAS gene in 101 EC and 5 EH samples.

The mutational frequency was 16 / 101 (15.8 %) in EC cases and 2 / 5 (40 %) in EH cases. None of eight nonendometrioid EC samples contained a mutation and have not been included in the following comparisons. In EC, KRAS mutation was associated with grade one (6 / 18; 33.3 %; p = 0.04) and was more frequent in patients staged I/II (15 / 82; 18.3 %) and in tumors that had not infiltrated the myometrium (3 / 12; 25 %) compared to patients staged III/IV (1 / 11; 9.1 %) and tumors that had invaded the myometrium (13 / 81; 16 %).

We conclude that KRAS mutations arise very early in the pathogenesis of EC and that they may contribute to the development of a large proportion of low grade, low stage endometrioid endometrial cancer. As the diagnosis of EH is complicated and has often been upstaged to high-grade EC following hysterectomy, the implications of KRAS mutational analysis should be considered.

**P06.207****Sensitive detection of KRAS mutations using mutant-enriched PCR and reverse-hybridization teststrips**

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The KRAS gene encodes a GTPase, which plays a vital role in cellular signaling processes. Mutated forms of the gene are potent oncogenes and found in many human cancers. KRAS mutations are also predictive for the response to cancer therapy with certain anti-EGFR monoclonal antibodies and tyrosine kinase inhibitors.

We have developed a reverse-hybridization StripAssay targeting 10

mutations in codon 12 and 13 of the KRAS gene. The test is based on PCR in the presence of a wild-type KRAS suppressor (mutant-enriched PCR), followed by hybridization of PCR products to teststrips presenting a parallel array of allele-specific oligonucleotide probes. The performance of the StripAssay was evaluated on DNA obtained from cultured cell lines, from formalin-fixed paraffin-embedded (FFPE) tissue and from stool. Using serial dilutions of DNA from various KRAS-mutated tumor cell lines into normal DNA, each of the 10 mutations was shown to be detectable at levels as low as 1%.

DNA samples containing various proportions of mutated KRAS were analyzed by the StripAssay in direct comparison to real-time PCR, dideoxy sequencing and pyrosequencing. While all methods correctly identified samples containing 25% mutated DNA, dideoxy sequencing and pyrosequencing failed to detect levels of 12.5% or lower. Both the StripAssay, as well as real-time PCR, unambiguously identified 10%, 5% and 1% of KRAS-mutated DNA in the presence of excess wild-type DNA.

The simultaneous detectability of 10 different mutations with excellent sensitivity will make the StripAssay a very useful tool for the assessment of the KRAS mutation status in cancer patients. (oberkanins@viennalab.co.at)

#### P06.208

#### A detailed analysis of *K-ras* point mutations in Russian patients with sporadic adenomatous polyps and adenocarcinomas of the colorectum

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To investigate spectrum, frequency and clinical significance of *K-ras* point mutations in colorectal (CR) adenocarcinomas and polyps of Russian patients we examined alterations at codons 12 and 13 in primary sporadic adenocarcinomas at various stages and differentiation from 58 patients, in adenomas with different histology and displasia from 33 patients, and in malignant polyps from 13 individuals. The average age of patients with cancer was 63.2, with adenomas - 58.8, and with malignant polyps - 62.1. DNA was extracted from surgical material. The mutations were studied using PCR, SSCP, RFLP and automatic sequencing. 25 (43.1%) of carcinomas, 16 (48.5%) of adenomas and 9 (69.2%) of malignant polyps examined harbored

*K-ras* mutations. The mutation pattern of *K-ras* of CR carcinomas was GGT->GAT (32%), GTT (16%), GCT (12%), aGT (8%), tGT (8%), GGC->GAC (20%), cGC (4%); in adenomas - GAT (37.5%), GTT (31.3%), aGT (6.2%), cGT (6.2%), GaC (18.8%); and in malignant polyps - GAT (33.3%), GTT (11.1%), tGT (22.3%), GaC (33.3%). Thus the mutation profiles of *K-ras* at codons 12 and 13 in Russian patients are not different from spectrum found in other parts of the world. The relationship between the presence of *K-ras* mutation in samples and clinicopathological data of the investigated individuals (age at diagnosis, sex, staging of cancer, histology of adenomas, the location of tumours or polyps) were analysed.

### P07. Cancer cytogenetics

#### P07.01

#### High-resolution mapping of chromosomal rearrangements at common fragile sites on chromosome 14 in tumor cells

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Common fragile sites (cFS) are regions of genomic instability that are particularly prone to breakage under conditions partially inhibiting DNA synthesis. These regions are found in all individuals and appear to be the hotspots of chromosomal rearrangements in cancer and neurological diseases. Approximately 90 cFS regions have been cytogenetically identified, but only few of these have been determined at DNA sequences level and completely characterized. Two aphidicolin-inducible cFS on chromosome 14, *FRA14B* and *FRA14C*, are listed in Genome Database and located at 14q23 and 14q24.1, respectively. To identify the precise genomic position of these cFS, we have performed six-colour FISH-mapping with BAC-probes on metaphase chromosomes

of lymphocytes treated with aphidicolin to activate breakage at cFS. We have determined that *FRA14B* and *FRA14C* span large genomic regions of 600 kb and 800 kb, respectively. To assess the possible role of these two cFSs in cancer chromosome rearrangements we used fine-tiling oligonucleotide array CGH with subsequent validation of obtained results by PCR and FISH. We have detected multiple breakpoints within *FRA14B* and *FRA14C* occurring in cancer samples from breast, colon and neuroblastoma. Non-random distribution of breaks along chromosome 14 with preferential involvement of cFS regions was demonstrated in different cancer cell lines and primary breast tumors.

#### P07.02

#### Cytogenetic Effect in Liquidators the Accident at Chernobyl NPP

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Objectives: To examine correlative relationship between radiation effect rate cytogenetic and clinical effects in those who participated in liquidation of the consequences of the accident at Chernobyl NPP during early and remote terms.

Methods: cytogenetic (analysis of chromosomes aberrations in culture of human lymphocytes) and statistic (model of spline regression). Group of researched was 12 100 liquidators.

Results: The work is aimed at the improvement of biologic (cytogenetic) dosimetry and indication of radiation affection rate in victims of radiation. Approximation of dose-effect dependence has been elaborated on the basis of chromosomes affection values in culture of human lymphocytes and model of spline regression. The proposed model differs from the other, based on traditionally used in biologic dosimetry linear and linear-quadratic models, in more accuracy of approximation and possibility to predict the effect of transition of calibrating curve on the plateau.

Inverse relationship of frequency of malignancies in liquidators of the consequences of the accident: the highest values are noted at low doses (10-50 mGy). Frequency of malignancies decreases with increase of radiation doses. The obtained data gave grounds for the hypothesis that low doses of absorbed radiation are statistically significant factor of carcinogenic risk. A conclusion was made that it can be connected with insufficient switching on anticarcinogenic defense of human organism at a range of low doses. Cytogenetic criteria for formation of groups of for formation of groups of increased cancer risk are determined.

Conclusion: Low doses of absorbed radiation are statistically a significant factor of carcinogenic risk.

#### P07.03

#### Array-based comparative genomic hybridization profiling of diffuse astrocytomas for genomic aberrations linked to prognosis

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The clinical course of patients with diffuse astrocytomas of WHO grade II (All) is highly variable. To identify genomic alterations possibly linked to prognosis, we screened 11 All from patients with a long recurrence free interval (RFI) of 60-144 months (All-long), 9 All from patients with a short RFI of 2-25 months (All-short), and 12 anaplastic astrocytomas (WHO grade III, AAIII) for genomic imbalances by genome-wide array-based comparative genomic hybridization. The number of genomic imbalances was higher in All-short as compared to All-long, with an average of  $9.7 \pm 1.6$  vs.  $7.5 \pm 2.4$  (mean  $\pm$  SEM) alterations per tumor

(range: 3-19 vs. 2-31), and highest in AAIII (mean $\pm$ SEM: 14.3 $\pm$ 3.3, range: 2-47). The following imbalances were identified to be frequent in AAIII and more frequent in All-short as compared to All-long: Gain on 7q (AAIII: 67%, All-short: 67%, All-long: 45%), loss on 10q (AAIII: 50%, All-short: 44%, All-long: 18%), loss on 14q (AAIII: 50%, All-short: 33%, All-long: 18%), loss on 19q (AAIII: 58%, All-short: 44%, All-long: 27%), gain on 20p (AAIII: 42%, All-short: 33%, All-long: 18%), gain on 20q (AAIII: 42%, All-short: 22%, All-long: 9%), and loss on 22q (AAIII: 33%, All-short: 22%, All-long: 0). In summary, All from patients with short RFI seem to contain a higher average number of genomic imbalances per tumor than All from patients with a long RFI. Furthermore, our data suggest that the presence of gains on 7q, 20p and 20q as well as losses on 10q, 14q, 19q and 22q may be linked to poor prognosis in All.

#### P07.04

#### Detection of a High incidence of Chromosomal Rearrangements in the Centromeric Regions of gastric adenocarcinoma subjects from Coimbatore south India

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Gastric cancer is the third most frequent type of neoplasia and the second most important cause of cancer-related death in the world. To evaluate chromosomal aberrations implicated in gastric carcinogenesis, we analyzed 24 samples of gastric adenocarcinoma by fluorescence in situ hybridization using a chromosome 8 a-satellite probe and by direct chromosomal analysis techniques. Trisomy 8 was the main finding of this study, observed in all cases. There was no significant difference between chromosome 8 ploidy and localization, stage, or histological type of adenocarcinoma in the experimental subjects. The high incidence of alterations we found in chromosome 8 may be a regional characteristic, related to the high incidence of this neoplasm in gastric adenocarcinoma and a strong influence of external factors, such as food habits. This aberration may comprise a cytogenetic subgroup of this neoplasm. Additional investigations are necessary to confirm the involvement of chromosome 8 and to identify genes in this chromosome related to gastric carcinogenesis. An increased copy number of chromosome 8 needs to be better investigated in other stages of gastric neoplasias, to clarify whether it is an etiologic cause of malignant transformation or a consequence of the proliferation process.

#### P07.05

#### The development of HPV cervical cancer is associated with the involvement of multiple genes

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Introduction: Cervical cancer is a worldwide disease and infection with high-risk human papillomavirus(HPV) is the major risk factor of its development.

Aim Of The Study: In cervical cancer of HPV infected women the ATM,p53,EGFR,HER-2/TOP2A genes,are involved and affect the outcome of the disease.

Recently two human telomerase gene locus, the hTERT(5p15.2) and hTERC(3q26) play a critical role.

Patients: Twenty women 22-51ys were transferred to Cytogenetic unit. All they had a family history of breast/ovarian cancer and they had a chronic cervical inflammation.

They got a new PAPtest looking for the presence of oncogenic HPV infection.

Methods: Peripheral blood lymphocytes(PB) were cultured using standard techniques.Thirty GTG banded metaphases were analyzed (ISCN2005). For FISH we used 2 panel probes(1)LSI HER-1,HER2/TOP2A/ CEP17,LSI p53,ATM(VYSIS).

(2)hTERT(5p15)/EGFR(5q31) and hTERC(3q26),C-MYC(8q24),SE triple color probe(KREATECH).

Two hundred interphase nuclei were counted for any single probe in all.

Results: High risk HPV 16/18 was detected in the 2youngest pts, the intermediate risk 35/39 in 10 and the low risk type 6/11 in 8.

The karyotypes looked normal.

In all there were circulating cells with amplification of EGFR,HER-2/TOP2A gene, and deletion of p53/ATM gene.

From the 2<sup>nd</sup> panel of probes there was amplified C-MYC,3q26 and 5p15.2 region.

Conclusions: (1)The presence of amplified genes in the **PB lymphocytes** suggest that there are malignant circulating cells.

(2)During their 2ys follow-up none has developed a definite cancer so a permanent oncogenic stimulus or other co-factors are required for neoplastic transformation.

(3)The gains of 5p15.2 and 3q26 are interesting given the presence of the hTR and hTERT genes, which can be the target for amplification during the transformation of human malignancies.

#### P07.06

#### Mosaic isochromosome Xp in a girl with short stature and bone marrow failure

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A 18-month girl, second daughter of unrelated parents, was diagnosed with bone marrow (BM) failure and treated with cyclosporine ad steroids, with partial response. At 6 years of age, she was referred to us for anemia, low white blood cell and platelet counts. BM aspirate showed no blasts or myelodysplasia; bone biopsy showed reduced cellularity and megakaryocyte number. Her height was <3rd centile due to partial GH deficiency. MR showed Chiari I malformation. Slight somatic dysmorphisms were observed. DEB tests on peripheral blood (PB) lymphocytes and fibroblasts were negative. c-Mpl gene associated with amegakaryocytic congenital thrombocytopenia, is not mutated. The Q-banded karyotype of PHA-stimulated PB lymphocytes was 46,X,i(X)(p10)[6]/46,XX[43]. Dual color FISH analysis using Xp- and Xq-arm partial paints confirmed the staining of the entire two isochromosome p-arms and revealed a subtle q-arm signal in the centromeric region. Compared to the normal X, the size of centromere signal (DXZ1 coupled with STS Xp22.3) was larger, suggesting an isodicentric chromosome. FISH with subtelomeric Xpter (RP11-215A12) probe and RP13-216E22 BAC probe, which specifically hybridizes to the XIST locus on Xq13.2, showed the absence of the inactivation center on i(X)(p10). Subtelomeric Xpter FISH analysis on BM cells demonstrated three signals in 11/300 nuclei. Fibroblast karyotype was 46,XX. Uncommon acquired i(X)(p10) associated with hematologic disorders as well as one case of constitutional mosaic i(X)(p10) in a girl with failure to thrive and thrombocytopenia have been reported in the literature. Our case seems to confirm the i(X)(p10) involvement in bone marrow failure.

#### P07.07

#### Alterations in p16 and p53 genes and chromosomal findings in patients with lung cancer: FISH and cytogenetic study

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Cromosomal aberrations and genes instability are two factors related to the genetic instability of cancer cells. A loss of tumor suppressor function of p16 and p53 are the most common event leading to the development of human cancers. Chromosomal abnormalities with lung cancer may provide a valuable clue to the identification of target loci and successful search for major genes. The aim of this study was to investigate alterations of p16 and p53 genes and chromosomal aberrations in patients operated on for small cell and non-small cell lung cancer by fluorescence in situ hybridization (FISH) and cytogenetic studies. We performed cytogenetic analysis by G-banding in 18 cases. FISH with p16 and p53 gene probes were used on interphase nuclei to screen the lung. We observed a high frequency of loss of p16 gene in 44% and p53 in 39% of our cases with LC. Structural aberrations predominated and usually consisted of deletions, breaks and fragilities of various chromosomes. Chromosomes 3 and 1 were found to be most frequently involved in structural abnormalities followed by chromosomes 6, 9 and 8. Otozomal aneuploidies were also observed to be most frequent. Expressed FSs were a significantly higher frequency of seven FS at 3p14, 1q21, 1q12, 6q26, 8q22, 8q24 and 9q13. Our data confirmed that DNA damage and genomic instability may be contributing factors to the mutation profile and development of lung cancer. The smokers who developed lung cancer also express a high frequency of the loss of p16 and p53, and the chromosomal aberrations.

## P07.08

## Molecular cytogenetic study of oligodendroglial tumors

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In oligodendroglial tumors, loss of genetic material on chromosomes 1p and 19q has been shown to predict better therapeutic response and longer survival. However, the significance of additional chromosomal aberrations has not been considered.

For detection of the 1p36 and 19q13 deletion and other chromosomal rearrangements in oligodendroglial cells, we performed dual-color interphase fluorescence in situ hybridization (I-FISH) with locus-specific DNA probes (Abbott Laboratories), 200 isolated whole cell nuclei (prepared from fresh non-fixed tumor tissue) were analyzed for each probe.

We examined 35 patients with histologically confirmed oligodendroglial tumors (10x oligodendrogloma, 19x anaplastic oligodendrogloma, 6x anaplastic oligoastrocytoma). Deletion of 1p36 and 19q13 region was detected in 28 cases (80%). In 12 of them combined deletion was found as a sole cytogenetic abnormality. Median of progression free survival (PFS) in this group was 47 months and only one patient died. In other 16 cases additional chromosomal rearrangements typical for high-grade gliomas were proved. In these patients significantly worse PFS was conferred (27 months, 6 patients died).

Genetic studies increase our understanding of oligodendroglomas. Although, deletion of 1p/19q was shown as a powerful favourable prognostic marker, our study demonstrates that prognosis is influenced by additional chromosomal aberrations. Further comprehensive whole-genome analyses of larger series with sufficient follow-up are needed to prove real prognostic significance and nonrandomness of these aberrations.

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## P07.09

## Retinoblastoma in monozygotic twins due to deletion of Rb gene

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Retinoblastoma (Rb) is the most common malignant tumor of the developing retina in children and occurs between 1 in 13,500 and 1 in 25,000 new born. In general, diagnosis is done at age of 12 month in bilateral cases and at age of 18 months in unilateral cases. Leukocoria, strabismus, unilateral mydriasis and heterochromia are the presenting clinical characteristics. Sixty percent of cases are nonhereditary and unilateral, 15 percent are hereditary and unilateral and 25 percent are hereditary and bilateral. There are few cases of monozygotic twins with retinoblastoma. In the present study we analyzed monozygotic twins with retinoblastoma in which only one of them was affected. DNA from leukocytes was obtained with conventional methods. Monozygosity was confirmed through GeneScan. Cytogenetic analysis was performed with conventional methods. FISH analysis was done with the cDNA LSI RB1 probe in leukocytes and oral cells of both twins and their parents and in retinal tissue of affected twin. FISH analysis in 500 cells of affected twin showed: a) two copies of the RB1 gene in 20% of retinal tissue and in 92% of leukocytes; b) one copy of the RB1 gene in 56% of retinal tissue and in 8% of leukocytes and c) no copies of the RB1 gene in 24% of retinal tissue. FISH analysis in leukocytes and oral cells (500 cells) of both parents and the non-affected twin showed no deletion of 13q14. We concluded that mutation in the twin with Rb occurred in a post-zygotic event.

## P07.10

## Value of combined array CGH and conventional cytogenetic analysis for a precise characterization of childhood and adult solid tumors

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Genetic information has become important to pathologists for the differential diagnosis of solid tumors. Chromosomal aberrations indicating an unfavorable prognosis are of clinical value. Microarray Comparative Genome Hybridisation (arrayCGH) allows for the detection of copy number changes throughout the entire genome at a resolution that far exceeds that of cytogenetics. ArrayCGH studies, especially of pediatric solid tumors, are still scarce.

We have analysed 48 patients using array CGH, conventional cytogenetics combined with FISH and, where possible, M-FISH. We have correlated our findings with standard morphological histopathological analysis and clinical features.

With this combined approach we have detected non random aberrations involving 1q and 2q, +8, +20, a t(1;6)(q21;q26) and a der(4)t(1;4)(q25;q34) characterising hepatoblastomas; 1q gain, del(1p), monosomy 22, aberrations involving 16(q13-qter), i(7)(q10) and a previously not published t(2;8)(p1.5;q22.3) in neuroblastomas (Wilms) tumors. ArrayCGH has been useful in the detection of karyotype abnormalities that were not detected by conventional cytogenetics, some of them having prognostic impact. Samples that have failed to grow in culture, for example osteosarcoma, or with a seemingly normal karyotype, could be analysed by arrayCGH with valuable results. Our findings bring further evidence regarding typical hepatoblastoma abnormalities, so far scarcely reported, with array CGH analysis refining the resolution of conventional cytogenetics. The detected changes in the series of neuroblastoma patients support the already existing evidence concerning prognostic significance of chromosomal aberrations in this tumor. Array CGH analysis may broaden the spectrum of detected changes and provide results even in negative or difficult to grow in vitro samples.

## P07.11

## Analysis of cancer genes in histopathologically tumour-free surgical margins in patients with oral squamous cell carcinoma

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Oral cavity cancer is the sixth leading cancer worldwide and more than 90% of malignant neoplasms of the oral cavity are squamous cell carcinomas. 10-30% of patients with oral squamous cell carcinoma (OSCC) develop local recurrences despite seemingly adequate tumour resection and the incidence of metastasis is high. As p53, c-Erb and c-Myc mutations have been considered as molecular changes typical of cancer cells, their presence, if any, in histopathologically free tumour margins, could be of great importance for the recurrence risk evaluation. The aim of this study was to determine the incidence of p53, c-Myc and c-Erb mutations in tumour margins, considered normal from a histologic point of view. DNA was obtained from 40 mucosa specimens, confirmed by a pathologist as free of neoplastic cells. P53 mutations were studied by PCR-SSCP, and sequencing, while differential PCR was used for the detection of c-Myc and c-Erb B2 amplification. A relatively high incidence of genetic lesions was detected: 11 out of 40 patients (27.5%) harboured p53 mutations, 5 (12.5%) had c-Erb B2, and 7 (17.5%) had c-Myc amplification. A close follow-up of these patients is planned.

Local and distant spread has been a major challenge for a successful cancer treatment. It is considered that verification of tumour-free resection margins by conventional histopathological examination is not sufficient. Molecular analysis of margins, targeting cancer genes, could enable the selection of OSCC patients at higher risk for tumour recurrence.

**P07.12****Screening of TERC gene amplification as an additional genetic diagnostic test in detection of cervical pre-neoplastic lesions**

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The aim of the current study was to present TERC gene amplification as possible diagnostic marker for use in routine cytological screening to improve the accuracy of conventional screening procedure in detection of cervical pre-neoplastic lesions.

Cervical smears were screened and classified as low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL). From the same specimens FISH procedure using TERC-specific DNA probe was performed. Copy number enumeration for TERC gene was evaluated. More than 2 signals per cell were considered as TERC positive case. In cervical smears graded after conisation as CIN1, no TERC positive cases were found in either LSIL or HSIL. Neither was TERC amplifications found in LSIL cases with histological results CIN1 and CIN 2. Amplifications of the TERC gene first appeared in HSIL cases with CIN2 histology. In the group of CIN3, TERC positive cases were present in LSIL and HSIL. In these, there were no statistically significant differences between TERC positive and TERC negative cases. Statistically significant differences in TERC positive cases were found between LSIL and HSIL without regard to the CIN grade.

From the results obtained, it can be concluded that TERC gene amplifications inevitably lead to a high risk of CIN3 in both LSIL and HSIL after cytological smear examination. A high CIN is not necessarily correlated with TERC amplification, but a positive TERC result certainly demands a high CIN classification.

**P08.01****Contribution of ACE I/D polymorphism to the risk of end stage renal disease in Romanian population**

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Introduction: End stage kidney disease (ESRD) is due to diabetes, hypertension, glomerulonephritis and cystic kidney disease. All this diseases seems to be connected with a genetic background which is predisposing to the development of the disease. Because diabetes and hypertension are the first two causes for ESRD and rennin angiotensin system has a important role in pathogenesis of this affection, ACE polymorphisms, specially ACE I/D have been extensively studied.

Aim: The aim of the present study is to evaluate the contribution of ACE I/D polymorphism to the risk of development of ESRD in diabetic and non-diabetic Romanian population.

Material and method: Clinical data and blood sample were collected from 321 Romanian type 1 diabetic patients, after inform consent. The patients were divided in three groups 1-healthy controls (119 patients), 2-ESRD due to other causes except diabetes (119 patients), 3-ESRD with diabetes (83 patients). Genomic DNA was extracted from peripheral blood leucocytes using commercial kits and the ACE I/D polymorphism was assessed by PCR-RFLP.

Results: The sample population is in Hardy-Weinberg equilibrium. The DD genotype was found in 36,13% in non-diabetic subjects and 32,25% in diabetic subjects comparative with 31,09% in healthy controls. The ID genotype frequency was 49,57% in healthy controls, 48,75% in non-diabetic patients with ESRD and 53,01% in diabetic subjects with renal insufficiency.

Conclusions: The DD genotype does not seems to confer a high risk for renal insufficiency in diabetic patients (OR=1,068, CI=95%) and in non-diabetic patients (OR=1,253, CI=95%). The heterozygote genotypes had a smaller risk for ESRD.

**P08.02****Incorporating Quantitative Covariates into Simultaneous Localization of Two Linked Loci Using Affected Relative Pairs**

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Many dichotomous traits for complex diseases are often involved more than one locus and/or associated with quantitative biomarkers or envi-

ronmental factors. Incorporating these quantitative variables into linkage analysis as well as localizing two linked disease loci simultaneously could therefore improve the efficiency in mapping genes. Previously, we proposed a robust multipoint Identity-by-Descent (IBD) approach to estimate a disease locus using affected sib pairs with incorporation of a quantitative covariate. In the present study, we extended this approach to simultaneously estimate two linked loci using different types of affected relative pairs (ARPs). We showed the efficiency was enhanced by localizing two disease loci simultaneously and by using relative pairs than using affected sib pairs alone after incorporating a quantitative covariate through parametric or non-parametric modeling. In addition to help identify factors associated with the disease and to improve the efficiency in estimating disease loci, this extension also allows us to account for heterogeneity in risk ratios for different ARPs. The collaborative study on the genetics of alcoholism (COGA) data released for GAW14 was used to illustrate the application of this extended method. The quantitative variable "maximum number of drinks in a 24 hour period" was incorporated into the linkage mapping when searching for two linked disease loci simultaneously using affected relative pairs. This example illustrated that the efficiency in estimating disease loci was enhanced by incorporating a quantitative covariate, by using all relative pairs as well as by mapping two linked loci simultaneously.

**P08.03****Alcoholism and genetic polymorphisms of gabaergic system: an experimental SNPsStream study**

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Previous studies have advanced the hypothesis that gamma-aminobutyric acid (GABA) is associated to alcohol use disorder (abuse and dependence). This association is suggested by studies that link alcohol to withdrawal tolerance, and the symptoms that define alcohol use disorder. The aim of our study was to examine single nucleotide polymorphisms (SNPs) in the glutamate decarboxylase (GAD) 67 gene, the rate-limiting enzyme in the synthesis of GABA, associated with alcohol abuse.

The research has been structured as a case-control study. The total cohort analyzed was 283 individuals, 107 of which were alcohol dependent according to the DSM IV TR criteria and 176 controls recruited from blood donors. The study protocol has been approved by Ethical Committee.

Specifically we analyzed 26 SNPs localized in the coding and in the untranslated regions of the GAD 67 gene with the *GenomeLab SNPsStream Genotyping System*. Our preliminary results show a significant difference in genotype distribution of one SNP (rs 11542313) localized in the exon 3 of the GAD 67 gene that is responsible for a silent mutation HIS-HIS ( $p=0.0015$ ). In order to clarify the meaning of this association, further genetic analysis are being undertaken. In particular, we are investigating other genetic polymorphisms up and downstream from rs 11542313 that could interfere with splicing and/or GAD 67 mRNA stability.

**P08.04****Association analysis of SNPs in the APP, RUNX1 and Dyrk1A genes on chromosome 21 with late-onset of Alzheimer's disease in a sample of Mexican patients**

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Background: The  $\beta$  Amyloid precursor protein (APP), Runt-related transcription factor 1 (RUNX1) and dual-specificity tyrosine (Y) phosphorylation-regulated kinase 1A (DYRK1A) genes are strong positional and biologic candidates for late-onset Alzheimer disease (LOAD) sus-

ceptibility. These genes are located on chromosome 21q21.3-22.13, and showed linkage for LOAD. Additionally, recent studies indicate that variants in the promoter of the APP gene could up-regulate the APP gene expression and that DYRK1A could be a key molecule bridging between  $\beta$ -amyloid production and tau phosphorylation. Several polymorphisms of these genes have been analyzed, however a systematic meta-analysis of these SNPs in previous association studies, suggested that only (APP=rs364048), (RUNX1=rs4816501) and (DIRK1A= rs2835740) are true allele risk with OR>1.80. Materials and Methods: A case-control study was design to evaluate the possible association between these SNPs with LOAD in Mexican patients. We studied 58 patients with LOAD and 70 sex and age-matched controls subjects. We analyzed alleles and genotype distributions for APOE ( $\epsilon$ 2/ $\epsilon$ 3/ $\epsilon$ 4), (-955A/G) of the APP promoter, (C/T) of the RUNX1, and (T/C) of the DIRK1A. Results: We found different genotype frequencies for all SNPs analyzed between cases and controls. Association was observed for the APOE  $\epsilon$ 4 allele (OR =1.65), AA genotype of APP (OR =1.67), TT genotype of RUNX1 (OR=1.24) and CC genotype of DIRK1A (OR =1.24). Conclusions: These data suggest a genetic association between these genotypes with LOAD in Mexican population. Our results differ from studies performed in other populations. Acknowledgements: This work was supported in part by CONACyT (2004-C01-129) and UNAM (SDEI. PTID.05.5)

#### P08.05

##### Analysis of PIN1 genetic variation in Alzheimer's disease

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Background: Alzheimer's disease (AD) is one of the most common neurodegenerative disorders. As PIN1 plays a significant role in the brain and the influence of PIN1 genetic variants in Alzheimer's disease remains unresolved, we performed an exhaustive analysis of PIN1 in the Polish cohort.

Methods: 111 late onset (AAO: 73.2 $\pm$ 5.0 years, range 66-88; 69.4% females) and 49 early onset AD patients (AAO: 52.6 $\pm$ 9.8 years; 57.1% females) were recruited. The control group consisted of 104 healthy, non-demented individuals (mean age: 75.1 $\pm$ 5.2 years, range: 68-90; 71.15% females). We performed sequencing and/or dHPLC of PIN1 promoter and coding region in the studied groups and real-time PCE to quantify PIN1 expression in the patient with c.58+64C>T substitution. Results: Genotype, allele and haplogroup frequencies of common PIN1 polymorphisms were similar in LOAD and EOAD patients in comparison with the controls. However, we identified four novel PIN1 mutations (g.9805833T>C, c.24C>T, c.58+64C>T, c.382+105C>T) in early-onset AD patients (EOAD). c.58+64C>T substitution was also present in two late-onset AD patients (LOAD). None of the novel variants was detected in the control group.

Conclusions: Although analyzed common polymorphisms are not associated with AD in the Polish cohort, further studies are required to resolve whether rare PIN1 mutations could contribute to AD etiology.

#### P08.06

##### Variations in two lipid metabolizing genes and susceptibility to sporadic Alzheimer's disease

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Alzheimer's disease (AD) is the most common form of dementia. Currently, the apolipoprotein E (APOE)  $\epsilon$ 4 allele is the sole identified genetic risk factor for sporadic AD (sAD) but the underlying mechanism is not understood. One hypothesis to explain how the  $\epsilon$ 4 allele might affect increase AD risk in humans relies on an interaction between APOE and its receptors. Particularly, the low density lipoprotein receptor-related protein (LRP1) is the main APOE receptor in the brain. LRP mediates clearance of  $\beta$ -amyloid aggregates of which lead to the for-

mation of senile plaques, a pathological hallmark of AD. Consequently, any variations in LRP gene will lead to a defect in clearance of LRP high affinity ligands, as the LRP-associated protein gene (LRPAP1). Taken together, these data suggest that LRPAP1 could be involved in the clearance of  $\beta$ -amyloid, and genetic variation at this gene could modulate the rate of plaque formation. The association of the C766T polymorphism in exon 3 of the LRP gene with AD is discussed controversially. Here, we analyzed this LRP variation and the association of intron 5 LRPAP1 (37 bp insertion/deletion) polymorphism in a number of sAD patients and controls from the same white population (Southern Italy). No statistically significant differences were found in LRP1 and LRPAP1 genotype and allele frequencies between the AD sample and controls. Together, the findings do not support a strong correlation of the exon 3 LRP and intron 5 LRPAP1 polymorphisms with AD, indicating that these variations only represent a minor risk factor for AD.

#### P08.07

##### Detecting recessive loci using very distant consanguineous relationships in a case study (AMRF)

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We recently identified a novel epilepsy gene for Action Myoclonus Refusal Failure Syndrome using just three unrelated affecteds. Only one affected was known to be the offspring of a consanguineous relationship. We identified an additional consanguineous relationship, which was not known, for one of the other affecteds. Since publication of this gene we have identified mutations in a further ten patients displaying a total of ten different mutations in this gene.

Simplified Hidden Markov Models (HMMs) to identify identity by descent (IBD) sharing between distantly related individuals have recently been proposed. These methods can be viewed as halfway points between pedigree-free homozygosity by state sharing methods and full multipoint mapping and can assess sharing in very deep consanguineous loops due to the availability of the much higher density SNP chip mapping data.

These new approaches are promising but have not been applied to many case studies. We have applied these methods to our AMRF cohort where we know that the mutations lie in the gene SCARB2. This allows assessment of their potential in identifying new susceptibility loci for rare recessive diseases genome wide with just single cases known, or inferred to be, the offspring of distant consanguineous relationships.

#### P08.08

##### ACE I/D polymorphism is associated with abdominal aortic aneurysm

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The purpose of this study was to examine the relationship between the polymorphism in angiotensin converting enzyme gene (ACE, I/D) and abdominal aortic aneurysm (AAA), a common vascular disease with high fatality rate. Previous studies have already pointed out that the genes of the RAS system may have a significant role in AAA formation.

The study included a total of 127 unrelated individuals: 63 patients with AAA (56 males and 7 females, median age 69, range 32-84), and 64 healthy controls comparable for sex and age. Out of 63 patients who underwent surgery, 33 were with ruptured aneurysms (RA) and 30 with nonruptured, asymptomatic aneurysms (NRA). They were genotyped for the ACE I/D polymorphism by standard PCR analysis. Allele and genotype frequencies were compared between patients and controls using Chi-square test, and Kruskal-Wallis test was used to examine differences in aneurysm diameter between genotypes.

ACE D allele was significantly more frequent in AAA patients compared to the healthy controls (0.722 vs. 0.578, p=0.016). The genotype distribution of ACE I/D was significantly different between patients and controls, but not between RA and NRA group. Significant association was found between DD genotype and the presence of AAA (OR DD

vs. ID+II= 2.897, 95% CI 1.409-5.955,  $p=0.003$ ). Average aneurysm diameter was largest in patients with DD genotype ( $82.46 \pm 3.3$  mm) compared to ID and II genotypes, but the difference was not statistically significant ( $H=4.71$ ,  $p=0.09$ ).

Conclusion: our results show significant association between ACE DD genotype and susceptibility to AAA.

#### P08.09

#### APOC3 Gene -482 C>T Polymorphism and Plasma Triglyceride Levels in a Turkish Population

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Coronary artery disease (CAD) is the leading cause of death in the United States and in Europe. Elevated plasma concentrations of triglycerides is an independent risk factor for CAD. APOC3 is a member of apolipoprotein gene family. Several studies demonstrated that -482 C>T single nucleotide polymorphism within the insulin responsive element in the promoter of APOC3 gene is associated with elevated plasma triglyceride levels.

We investigated the distribution of APOC3 gene -482 C>T polymorphism in 67 hypertriglyceridemic patients and 147 age and sex matched controls. Genomic DNA was extracted from whole blood by salting out method. The polymorphism was analyzed using polymerase chain reaction (PCR) - restriction fragment length polymorphism (RFLP) assay. Genotype and allele frequencies and their associations with CAD risk, demographic factors, and smoking status were investigated.

The percentages of the CC, CT, and TT genotypes were 34.3%, 10.4%, 55% for the patients and 46%, 8.2%, 45.6% for the controls, respectively. There was no significant association between the APOC3 -482C>T polymorphism and plasma triglyceride levels in the studied population. (OR=1.39, 95% CI=0.89 - 2.18,  $P=0.131$ ).

#### P08.10

#### Genome-wide association of pulse wave analysis phenotypes in two isolated populations

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Pulse wave analysis (PWA) using the SphygmoCor system is a non-invasive applanation tonometry-based method of recording the pulse pressure waveform in a peripheral artery. The corresponding central pressure waveform can be derived from the measured radial waveform. The shape of this wave contains information on cardiac contractility and the size, speed and shape of wave reflection, which are in turn determined by factors like arterial stiffness. A number of PWA parameters including the augmentation index are associated with classical cardiovascular risk factors and events. We have measured a range of PWA phenotypes in samples from two island isolates; the Orkney Isles in Scotland (540 subjects) and the Croatian island of Korcula (480 subjects). All samples were genotyped at 318,000 SNP markers using Illumina beadchips. Association tests were performed separately in the two populations using a genomic kinship method to correct for the close relationships among participants. Meta-analysis of these data identified seven separate genomic regions showing novel associations (up to  $P = 3 \times 10^{-8}$ ), with one or more of the PWA phenotypes, including central augmentation pressure and time delay from incident to reflected wave. Replication has been sought in 3000 independent samples from the Anglo-Cardiff Collaborative Trial (ACCT). Several genes and potential regulatory regions which have not previously been associated with haemodynamic traits have been identified here and therefore warrant further study.

#### P08.11

#### Identification of genes regulated by disease associated SNPs in gene deserts

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Genome wide association studies in complex diseases such as asthma and inflammatory bowel diseases (IBD) revealed in many candidate chromosomal regions SNPs and haplotypes most significantly associated with disease are located in non-coding regions or even in "gene deserts" suggesting SNPs in non-coding regions play important role in complex diseases.

We have developed approach for identification of functional SNPs in cis-elements associated with allele specific expression in disease candidate regions. In this approach we initially correlated our gene expression data we have obtained from more than 500 lymphoblastoid cell lines from CEPH families to public released International HapMap project genotype data preformed on the DNA isolated from the same CEPH cell lines. We have confirmed genotype-gene expression correlation resulting from lymphoblastoid CEPH cell lines in blood lymphocytes isolated from patients trios using quantitative transmission disequilibrium test (qTDT). In addition we confirmed genotype-gene expression correlations in patients tissue biopsies where available. The SNPs most significantly associated with altered gene expression were used for disease association study in patients and controls. We have applied our approach to Slovenian IBD and asthma cohorts each consisting of more than 400 patients and matching healthy controls and among others confirmed PTGER4 gene as best candidate gene in 5p13.1 IBD candidate region.

#### P08.12

#### The LKB1-AMPK-TORC2 signaling pathway and its contribution with development of type 2 diabetes in Japanese

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The LKB1-AMPK-TORC2 signaling pathway controls glucose homeostasis in the liver, and mediates therapeutic effects on insulin sensitizing antidiabetic agents. We hypothesized that genetic polymorphisms of the *STK11*, *PRKAA2* (encoding AMPK  $\alpha_2$  subunit) and *CRTC2* (encoding TORC2) could influence the susceptibility to T2D. We screened exons, untranslated regions and exon-intron boundary of *STK11* and *CRTC2* and genotyped in 1787 Japanese subjects. Additionally, the previously described association between the *PRKAA2* haplotype and T2D was tested for replication. According to single locus association test, an intronic SNP in the *STK11* (rs741765; OR 1.33, 95% CI 1.05-1.67,  $p = 0.017$ , under a recessive genetic model), and a non-synonymous SNP in the *CRTC2* (6909C > T: Arg379Cys; OR 3.01, 95% CI 1.18-7.66,  $p = 0.016$ , under a dominant model) showed a nominal significant association with T2D. In *PRKAA2*, two non coding SNPs, rs1418442 (previously been reported to be associated with serum cholesterol in Caucasian females) and rs932447 were associated moderately with T2D (OR 0.62, 95% CI 0.40-0.96,  $p = 0.030$ , under a recessive model). Haplotype analysis showed that only in *STK11*, one haplotype containing the minor T allele of rs741765 was slightly associated with T2D ( $P=0.04$ ). The association of *PRKAA2* haplotype reported previously in Japanese was not replicated in our samples. Among the three genes investigated herein, gene-gene (SNP-SNP) interaction studies provided evidence for an interaction between *STK11* and *CRTC2* influencing susceptibility to T2D. In conclusion, we found a weak evidence that *STK11*, *PRKAA2*, or *CRTC2* polymorphisms contribute to the susceptibility to T2D in Japanese.

#### P08.13

#### On the value of family data in genome-wide association studies for quantitative traits

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GWAS of quantitative traits are typically done using population-based samples of subjects that are, most often, ascertained irrespective of their trait values or of the trait distribution in their relatives. It is well known, however, that greater power can be achieved in genetic pop-

ulation-based studies by selecting subjects from the tails of the trait distribution. Family-based study designs have not been the design of choice mainly because of the reduced power of the orthogonal-based association tests. Association testing in family data can be performed under alternative methods, as the Measured Genotype test that has been shown to outperform orthogonal-based tests. Therefore, association designs based on family data can prove to be of more value than assumed, especially for replicating and/or following-up results from population-based association results.

Here, our main aims are to evaluate the performance of association designs using either population-based data only or combining both population and family data in a multi-stage approach. A two-stages association study is designed. The first stage uses population data to identify the set of SNPs positively associated to the trait at a given nominal significance. These positive SNPs are tested for replication in a second stage that uses either population or family data. We developed a simulation study to estimate type I and type II error rates of these two association designs. The data were simulated under varying conditions according to (1) the criteria used to ascertain the (population or family) data; (2) the effect sizes of the functional variant; (3) trait heritability.

#### P08.14

#### Common variants of TAC3 and TAC4 tachykinin genes and susceptibility to asthma

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Asthma is a complex disease resulting from both genetic and environmental factors. Its pathological features involve an intricate interplay between different cell types including those from lung and also inflammatory and immune cells. In humans, tachykinins and their receptors are expressed in many of these cells and have been shown to be involved in airway hyperresponsiveness, bronchoconstriction, edema, and mucus secretion.

The aim of this study was to investigate a possible association between eight single nucleotide polymorphisms (SNPs) of two tachykinin genes, TAC3 and TAC4, and asthma susceptibility. A case-control study was performed in the Canary Islands population (Spain), where asthma prevalence has been estimated to be much higher than in continental populations. The investigation was conducted on 102 patients, with clinically defined asthma, and on 100 healthy subjects. Genotyping was done by multiplex polymerase chain reaction and a subsequent single base extension assay (SNaPshot®).

Nominal significance was observed for two SNPs, one in the TAC3 gene conferring asthma protection (Odds ratio [OR]: 0.46; 95% Confidence Interval [CI]: 0.22-0.97;  $p=0.038$ ), and another one in TAC4 associated with an increased risk for asthma (OR: 1.94; 95% CI: 1.06-3.54;  $p=0.03$ ).

Although this study is currently ongoing, these results suggest a possible role for common variants of the TAC3 and TAC4 genes in asthma susceptibility in the Canary Islands population.

#### P08.15

#### Meta analysis of filaggrin polymorphisms in eczema and asthma: robust risk factors in atopic disease

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and Allergy Biederstein, Technische Universitaet Muenchen, Munich, Germany. The discovery of null mutations in the gene encoding the key epidermal protein filaggrin (*FLG*) as a major risk factor for eczema and related asthma represents a milestone towards the understanding of an important genetic mechanism in these complex diseases. Studies published to date demonstrate differences concerning study design and strength of associations and reported conflicting results on the impact of *FLG* in asthma.

We conducted a meta-analysis of 24 studies on common *FLG* mutations and AE involving 6448 AE cases, 26787 controls and 1993 AE families as well as 17 studies involving 3138 cases, 17164 controls and 4 family studies including 1511 affected offspring on asthma. Odds ratios (OR) for case-control studies ranged from 1.69 to 11.56 with an overall OR of 3.39 (95%CI=2.73-4.23), while family studies showed more homogeneous results. *FLG* mutations are also significantly associated with asthma with an overall OR of 1.48 (95%CI=1.32-1.66). While strong effects for the compound phenotype "asthma + AE" with an OR of 3.29 (95%CI=2.84-3.82) were observed, there appears to be no significant association with asthma in the absence of AE.

This meta-analysis summarizes the strong evidence for a high risk for AE conferred by *FLG* null mutations, and refines the risk profiles of *FLG* alleles suggesting an association with both more severe disease and a dermatologist diagnosis. The results clearly indicate that *FLG* null alleles are a robust risk factor in determining genetic predisposition to asthma, and suggest that *FLG* deficiency might help define the endophenotype of asthma linked with eczema.

#### P08.16

#### Glucocorticoid receptor gene polymorphisms associated with bronchial asthma in children

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**Objective:** The authors hypothesized that glucocorticoid therapy efficacy might be associated with polymorphisms in the glucocorticoid receptor (GR) gene. We compared clinical course of asthma among children with different genotypes of Bcl1 and Tth111I polymorphisms of the GR gene. In children with difficult asthma we investigated exon 2 of the GR gene by sequencing.

**Methods:** Study group consisted of 485 children (age 2-17) with asthma; control group consisted of 151 healthy children. The Bcl1 and Tth111I polymorphisms were detected by PCR-RFLP. We studied exon 2 by sequencing in group of 24 patients with difficult asthma.

**Results:** We not found differences in allele and genotype frequencies of Bcl1 variant in asthma patients and controls. Genotype TT of Tth111I polymorphism was rare in girls with asthma compared to boys with asthma and healthy girls.

However, the clinical characteristics of patients (severity of exacerbation, tests for bronchial hyperreactivity, lung function data, IgE levels, dynamics of clinical symptoms, level of asthma control via inhaled GC) showed that children carrying Bcl1 CC genotype and Tth111I CC genotype have more mild clinical presentations of asthma than G-allele or T-allele carries. We found that genotype CT of Tth111I polymorphism was more frequent in 24 children with difficult asthma compared to children with severe asthma.

Among 24 children with difficult asthma 3 previously reported polymorphisms (198G>A, 200G>A, 1220A>G) were found in exon 2.

**Conclusion:** GR gene polymorphisms linked with levels of asthma severity and inhaled glucocorticoids therapy efficiency in children with asthma.

#### P08.17

#### TGF-β1 and TGF-β3 are upregulated in lung of toxic inhaled patients, and improve effeocytosis and survival time

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During Iraq imposed war on Iran, thousands of Iranians were exposed to toxic inhalants, and survived people suffer from respiratory disease, Bronchiolitis Obliterans (BO). Most recently it has been indicated that

TGF- $\beta$  improve the efficiency of efferocytosis (engulfment of apoptotic cells by phagocytes followed by cell replacement to maintain homeostasis) in lung, and several lung diseases, including Asthma, Chronic Obstructive Pulmonary Disease(COPD) and Cystic Fibrosis (CF) result from impaired efferocytosis. In order to clarify the significance of each of TGF-  $\beta$  isoforms in lung disease of people poisoned by toxic inhalants, we examined the mRNA expression for TGF-  $\beta$  1, TGF-  $\beta$  2 and TGF-  $\beta$  3 in lung biopsies of chemical injured patients, and compared it with non-injured people. Lung samples were collected from both groups using small pinchers attached to a long cable threaded through the bronchoscope. Total RNA was extracted, cDNA libraries were constructed, and Semiquantitative RT-PCR was performed using GAPDH gene as internal control. Our result indicated that level of TGF-  $\beta$  1 and TGF-  $\beta$  3 mRNA was significantly higher in chemical gas injured patients than non-injured group ( $p<0.05$ ). The expression of TGF-  $\beta$  2 gene was too low to be quantified by RT-PCR method in both of the groups. Hereby we suggest that TGF- $\beta$ 1 and TGF- $\beta$ 3 but not TGF- $\beta$ 2 may improve the efferocytosis and play a role in airway remodeling and lung homeostasis in chemical injured group. These properties of TGF- $\beta$  are consistent with long time survival of chemical injured people suffering from BO.

#### P08.18

##### A genome wide search for genetic determinants of fibrinogen levels reveals a candidate gene on chromosome 12 and a new sex-related locus on chromosome 17

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Introduction: High plasma levels of fibrinogen are well-established risk factors for cardiovascular disease. These levels are influenced largely by genetic factors, with a heritability ranging from 20-40%. However, only some polymorphisms in the fibrinogen structural genes have been reported, which explain a small proportion of the genetic variability.

Material and methods: we performed a Genome Wide Scan (GWS) for fibrinogen levels in 21 families from the GAIT project using 500 microsatellites scattered throughout the genome. We fine-mapped 2 regions that showed a clear linkage signal with fibrinogen levels with extra SNPs. (1.195 SNPs on Chromosome 12, and 667 SNPs on Chromosome 17). Analyses were performed in male and female separately.

Results: We found one significant linkage signal on chromosome 12 (LOD=2.1; nominal  $p=0.00094$ ), and one on chromosome 17, only detected in females (LOD=3.2; nominal  $p=0.00003$ ). 2 SNPs, localized on the *TCF1* gene, showed a statistically significant association with fibrinogen levels on chromosome 12. This gene codifies for Hepatocyte Nuclear Factor 1 implicated on transcription of alpha and beta chains of fibrinogen. 8 SNPs have shown a statistically significant association with fibrinogen levels on chromosome 17.

Conclusion: Looking at these results, *TCF1* is postulated as the responsible gene for the linkage signal on chromosome 12. On chromosome 17, we have enclosed a 10 Mb region which contains several interesting hormone-related genes which could explain the female-specific signal in this region.

#### P08.19

##### Selection and migration as possible causes of failure of case-control study replications

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Replications of genetic association studies for human disease often give variable results. Stimulated by a recently described geographic cline in odds ratios, we investigated fifteen sets of at least seven replicates of the same allele-disease case-control studies and found a striking pattern: odds ratios generally decline with increasing frequency of the marker allele. The OR even flips from risk to protective in seven out of fifteen sets.

We investigated whether this could be a sampling artefact or the result of population processes, such as drift, selection or migration. Drift appears unlikely to have created these patterns. Both selection and migration can cause the observed pattern, but only in specific circum-

stances. When populations differ in allele frequency and disease prevalence, population admixture could occur, but we could not find how this would cause such a general pattern.

Exploration of existing SNP datasets confirmed that no general processes of drift and migration of European populations produce such patterns. This suggests that disease alleles experience specific processes that do not apply to the majority of random SNPs

The observations should warn investigators that non-replication or even a reversal of the OR is possible -especially if the allele frequency of the marker in controls is larger than that of the original publication- and need not invalidate the initial observation.

#### P08.20

##### Do HOXB9 and COL1A1 genes play a role in congenital dislocation of the hip? Study in a Caucasian population

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Congenital dislocation of the hip (CDH), which is one of the most common congenital skeletal disorders, corresponds to an abnormal seating of the femoral head in the acetabulum. It is commonly admitted that CDH presents a genetic component. However, little is known about the genetic factors involved. This study aimed to determine the role of two potential candidate genes on chromosome 17 in CDH: HOXB9 (involved in limb embryonic development) and COL1A1 (involved in joint laxity). We set up a case-control association study (239 cases and 239 controls) in western Brittany (France) where CDH is particularly frequent. The set of informative single nucleotide polymorphisms (SNPs) in each gene was selected using Tagger and genotyped using the SNaPshot method ( $n=2$  and  $n=10$  respectively). The association was tested both through single-locus and haplotype-based analyses, using SAS and Haploview software. In addition, we carried out the transmission disequilibrium test (TDT) with the same polymorphisms from a sample of 81 trios (i.e. 81 patients included in the case-control study and their both parents). The case-control study revealed no significant association between CDH and the tagSNPs selected in both HOXB9 and COL1A1. Moreover, the TDT did not reveal distortion in allelic and haplotype transmission of the studied markers. Our study did not support an association between HOXB9 and COL1A1 and CDH in our population. These negative findings were obtained by population- and family-based designs. Analysis of the genetic component of CDH should focus on other candidate genes.

#### P08.21

##### Distribution of 35delG "silent" mutation in the GJB2 gene among healthy population in Northwest region of Russia

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Present literatures demonstrate that more than half cases of congenital hearing impairment with an occurrence of 1 in every 750-1000 newborns are associated with hereditary pathology, where Connexin 26 (GJB2) gene mutations seem to be the main molecular-genetic cause for the nonsyndromic heraring loss.

Of these, the most common cause of autosomal recessive hearing loss is 35delG mutation, which is responsible for 70% of prelingual hearing loss cases among caucasians. The average carrier frequency of 35delG among the healthy European population is about 1.26 % in Northern Europe and 2,85% in Southern Europe. Although GJB2 gene mutation has been widely studied throughout the world for over 15 years, Russia is still at the early stages of analyzing this mutation. Distribution of 35delG (GJB2) mutation among the population in the Northwest region of Russia was investigated in this study.

Presence of the 35delG mutation in the GJB2 gene was detected by PCR in 1153 healthy slavic origin individuals (603 male and 550 fe-

male), residing in northwestern cities of Russia (St. Petersburg, Pskov, Arkhangelsk and Kaliningrad), and a high frequency heterozygous carriers of this mutation was revealed (5,5%; 4,7%; 5% and 7,5% correspondingly).

This is the first study where the distribution of 35delG heterozygous carrier was analyzed among healthy Northwest population of Russia. The obtained data suggest that the distribution of «silent» mutation in the northwest region of Russia significantly exceed the known European data.

#### P08.22

#### Genotype and phenotype testing of CYP2D6 gene in 91 patient treated with Paroxetine

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The gene CYP2D6 encodes enzyme involved in biotransformation of psychotropics in psychiatric treatment. The Caucasian population has been grouped in according to the enzymatic activity as poor, intermediate, effective and ultrarapid metabolizers. The determination of metabolic activity prior the dose adjustment could be really useful in some cases.

The methodical approach of genotyping is based on long PCR and subsequent sequencing. These standard methods are combined with RealTimePCR and High Resolution Melting analysis (HRM) using Light Cycler 480 System. Recently is possible to detect the most frequent null alleles 3\* 4\* 6\* 7\* and 8\*. Allele 5\* could be detected by using agarose electrophoresis or UPL probes. HRM analysis has been performed for the most frequent SNP's in CYP2D6 gene as a fast cheap and reliable method for pre-genotyping.

O-demethylation of dextromethorfan was used for metabolic activity assessment. The concentrations of marker and metabolite in the urine were determined by HPLC assay. The phenotypes PM and EM were distinguished by using 0,3 antimode.

The clinical data were analyzed and compared with genotype and phenotype result.

This paper provides an overview of current technologies available for CYP2D6 genotype and phenotype testing and the result of testing of the group of 91 patients, treated with Paroxetine.

The effect of Paroxetine treatment on CYP2D6 is summarized in Table 1.

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Genotype	Phenotype	Number of patients	Frequency of phenotypes	MR>0,3 PM	MR<0,3 EM
*1*1	EM	31	50	55%	19 23
*2*1		8		1	7
*2*2		1		0	1
*1*9		2		0	2
*2*9		1		0	1
*1*41		2		0	2
*2*41		2		2	0
*1*10		2		0	2
*2*10		1		1	0
*1*4	IM	15	34 37,40%	7 21	8 13
*2*4		5		2	3
*33*4		1		1	0
*10*4		4		3	1
*41*4		1		1	0
*41*8		1		0	1
*41*3		1		1	0
*41*5		1		1	0
1*5*		5		5	0
*4*4	PM	3	7 7,70%	3 6	0 1
*5*4		2		2	0
*3*4		1		0	1
*3*5		1		1	0

#### P08.23

#### Role of Cytochrome P450 2C19 genetic polymorphisms on therapeutic efficacy of omeprazole in Iranian patients with erosive reflux esophagitis

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GERD is characterized by the reflux of stomach acidic contents to esophagus. Proton pump inhibitors (PPIs) such as omeprazole are the most effective agents available for the treatment of GERD. Cytochrome p450 2c19 (CYP2c19) enzyme is responsible for metabolism of omeprazole. Two common polymorphisms of cyp2c19 (G>A substitutions at codons 212, 227) decrease the enzyme activity. Individuals with different genotypes can be divided to extensive metabolizers (EMs) and poor metabolizers for omeprazole. This study was conducted to investigate the effect of cyp2c19 gene polymorphisms on omeprazole efficacy in relation to clinical and histological response to treatment in Iranian patients with reflux esophagitis (RE).

Eighty two Iranian patients with RE were enrolled in the study and underwent treatment with omeprazole 20mg/bd for 4 weeks. The grade of esophagitis was detected by endoscopy. Severity of symptoms, were assessed in the beginning and end of treatment. Cyp2c19 genotype was detected by RFLP method.

The rate of complete clinical response to treatment with omeprazole (20mg/bd) was 95 % ( 95% CI, 86%-103%) in heterozygous extensive metabolizer (hetero-EMs) group, that was higher as compared to the homozygous extensive metabolizer (homo-EM) group [43 % ( 95% CI, 32%-54%)]. (P<0.001) Cyp2c19 polymorphism influences the efficacy of omeprazole in Iranian patients with RE. The clinical response and endoscopic improvement of esophagitis are independent from each other in response to treatment with the same dosage of omeprazole, but they are both influenced by the cyp2c19 genotype status.

#### P08.24

#### TGF- $\beta$ codon 25 Polymorphism and the Risk of Graft-Versus-Host Disease after Allogenic Haematopoietic Stem Cell Transplantation

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Certain cytokine genotypes are associated with acute graft versus host disease (aGVHD) after bone marrow transplantation (BMT). The present study aimed to determine existing association between TGF- $\beta$ 1 codon 25 polymorphism and aGVHD after HLA-identical sibling BMT in the Iranian population. In a retrospective case-control study, 172 subjects including 86 Iranian HLA-identical sibling BMT donor/recipient pairs were recruited. All of the patients were affected by haematological malignancies (AML=40, ALL=25 and CML=21). PCR-SSP method was performed to determine TGF- $\beta$ 1 codon 25 G/C polymorphism genotypes. The frequency of TGF- $\beta$ 1 codon 25 GG, GC and CC genotypes among all subjects were 77.3%, 21.5% and 1.2% respectively. Recipients with the GG genotype developed severe aGVHD significantly more than those with CC or GC genotype (Odds Ratio =12.133, P=0.015). Genetic background of TGF- $\beta$ 1 may be involved in aGVHD development and/or severity in HLA-matched sibling BMT in Iranian population.

Key words: cytokine gene polymorphism • SNP • TGF- $\beta$ 1 • BMT • aGVHD

#### P08.25

#### Analysis of Dopamine receptor genes (D1- D5 ) expression on human peripheral blood Lymphocytes(PBL) in Rheumatoid Arthritis( RA)

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Recent studies reveal that immune cells can also synthesize catecholamines and that catecholamines can regulate immune functions. In addition, lymphocytes express the enzymes for synthesis of catechol-

amines and the enzymes for their degradation. These endogenous catecholamines synthesized by immune cells can regulate immune functions, including cellular proliferation, differentiation, apoptosis and cytokine production. In vitro, dopamine inhibits the proliferation of activated T cells and their cytokine secretion.

Changes in the dopamine system are influenced not only by dopamine itself, but also by dopamine receptors that are encoded by five different dopamine receptor genes (DR1-DR5). Metabolic abnormalities of catecholamines in immune cells could be related to autoimmune disease. Several observations indicate that catecholamines and their receptors may play a part in the pathogenesis of chronic inflammatory conditions, such as rheumatoid arthritis (RA). We have therefore asked whether changes in the dopaminergic system are associated with RA.

In the present study, we investigated dopamine receptor gene expression in PBMCs of 40 RA patients and 40 healthy individuals by RT-PCR. Quantitative gene expression analysis was made by real-time-PCR using primer pairs specific for the five dopamine receptors and beta-actin as internal control. We found that all types of dopamine receptors are present in lymphocytes of normal individuals and RA patients. However, a significant difference of DR2 and DR4 gene expression profiles was seen in RA, as compared to healthy individuals. We conclude that there is a quantitative significant difference of dopamine gene receptor expression in RA.

#### P08.26

##### Mutation analysis of the human fatty acid amide hydrolase gene and the risk of drug addiction

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Addiction to illicit drugs is a neurobehavioral disorder of complex origins. Although social and psychological factors contribute to addiction, genetic factors explicitly weigh in. To understand the role of genetic variations in vulnerability to drug addiction, the nature of population genetics has to be characterized by identifying the single nucleotide polymorphisms (SNPs) in candidate genes and performing association studies. Here, we investigated the SNPs in the coding regions of the fatty acid amide hydrolase (FAAH) gene which encodes the principal endocannabinoid-inactivating enzyme. A missense mutation in this gene, c.385C>A, has been reported to contribute to the functional alteration in the endocannabinoid system. Although a link between this polymorphism and drug abuse among white ancestral subjects has been reported, the specific allelic frequencies often vary in different populations. Hence, to explore this hypothesis in Malaysian subjects, we conducted a case-control study which included 80 drug addicts and 80 healthy controls. DNA sequencing analysis revealed a significant ( $p<0.01$ ) association between the 385A/385A genotype and drug addiction amongst Malaysian individuals. In addition, using high-resolution melt analysis several other novel SNPs within this gene were also uncovered. One of these novel FAAH SNPs resulted in the substitution of an amino acid residue in the Src homology 3 binding domain (SH3). Transfection experiments have demonstrated that this proline-rich region deleted amidase was enzymatically inactive. Collectively, these findings support the concept that genetic mutations in FAAH may constitute important risk factors for functional abnormalities in the endogenous cannabinoid system and drug addiction.

#### P08.27

##### ENPP1 gene variants have a strong impact for risk of type 2 diabetes but not on development of obesity in Japanese

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Previous studies have demonstrated the K121Q polymorphisms in the plasma cell membrane glycoprotein-1 (PC-1, recently known as

ENPP1) gene influences insulin resistance, obesity and risk of type 2 diabetes. However, conflict association results are reported in different populations. We previously found no evidence for association of K121Q polymorphisms with risk of type 2 diabetes and obesity in a Japanese population. This study for the first time was carried out in samples of Japanese to explore the key role of other previously reported polymorphisms and haplotypes containing K121Q variant with risk of type 2 diabetes. To accomplish this, we genotyped the rs997509, rs1799774 (IVS20delT-11) and rs7754561 (A/G\_1044TGA) polymorphisms for association analysis in 911 type 2 diabetic patients (459 female/452 male) and 876 control subjects (430 female/446 male) using TaqMan assay. According to single locus association test, in IVS20delT-11, we identified a significant association of delT allele with type 2 diabetes in Japanese (adjusted odds ratio, 1.50; 95% confidence interval, 1.15-1.99);  $p=0.0002$ . In a haplotype association analysis, we failed to find any significant association between Q-delT-G haplotype and type 2 diabetes ( $p>0.05$ ). A subanalysis of subjects depending on their body mass index (BMI) status revealed no significant impact of the 3 polymorphisms and Q-delT-G haplotype on obesity. In conclusion, our study indicated the potential role of an intronic polymorphism IVS20delT-11 with type 2 diabetes and no evidence of SNPs and haplotype association of ENPP1 gene with obesity in Japanese.

#### P08.28

##### MEFV genotyping for diagnostics and treatment of FMF

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FMF (Familial Mediterranean Fever) is characterized by recurrent, short (2-4 days) episodes of fever, accompanied by severe pain in the abdomen, chest or joints, and sometimes associated with erysipelas-like erythema. The most severe complication is progressive renal amyloidosis (RA). FMF is caused by a number of mutations in the MEFV gene located on chromosome 16p, encoding pyrin protein, which normally acts as a mediator in control of inflammation. Molecular genetic analysis significantly improves early and correct diagnosis of FMF, and allows to commence lifelong treatment of affected individuals with colchicine. Frequency of the mutations of MEFV gene is extremely high in Armenian population. We have reported about the molecular diagnostics of more than 10000 FMF patients in Armenians.

In our FMF patients the colchicine largely prevents the development of attacks and renal amyloidosis (RA). Adequate colchicinotherapy delays RA progression in FMF/RA patients. In a few cases the effect of colchicine remains controversial. We confirm that genotyping is assisting in prediction of the response to colchicine treatment. FMF homozygous patients for M694V mutation present a more severe phenotype and show a limited response to colchicine at a nephrotic stage of RA. In contrast, FMF patients with other genotypes still have a good chance to ameliorate the nephrotic syndrome and to maintain renal function.

**Conclusion:** According to our results, the genotyping is recommended for all siblings of FMF patients and to search for other autoinflammatory periodic fever syndromes among patients with specific clinical symptoms but without MEFV mutations.

#### P08.29

##### GAA Repeat Polymorphism and Cytogenetic findings in Turkish Friedrich's Ataxia Patients

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Friedreich ataxia (FA), a progressive heredodegenerative disorder, is one of the most common forms of autosomal recessive ataxias. FA is the result of a gene mutation at the centromeric region of chromosome 9 (9q13-21.1) which is the site of the gene encoding for the 210-amino-acid protein frataxin.

Results of GAA repeat polymorphism in 61 family members of 30 typical FRDA patients were reported. GAA triplet repeat size ranged from approximately 7 to 34 in normal alleles and from 66 to 1300 in mutant alleles. Forty six patients were homozygous for GAA expansion and size of expanded alleles differed from 425 to 1300 repeats. Children 2 and 6 years old of one family had homozygous GAA expansions reaching 925 repeats. All 30 families studied had at least 1 afflicted

child and 9 parents and 2 siblings were carrier (heterozygous) with mutant alleles ranging from 66 to 850 repeats. Family studies confirmed the meiotic instability and stronger effect of expansion in the smaller alleles on phenotype and a negative correlation between GAA repeat expansion size and onset-age of the disease. In additionally; In nineteen FA patients (11 males and 8 females) with the age range of 6 to 34 years (mean  $22.26 \pm 7.06$ ) and their relatives with the age range of 4-72 years (mean  $34.6 \pm 17.9$ ), cytogenetic studies were performed. Three of them had different cytogenetic findings which were 46,XY,Yqh+, 46,XX,t(7;15),(q34;q21), 46,XY,inv(9)(q21;p12).

#### P08.30

##### GABRG2 and ADH polymorphisms in alcohol dependence

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Our study aimed to investigate role of GABRG2 and ADH gene polymorphisms in patients who had alcohol dependence. In the literature, there are controversial results on the role of these gene polymorphisms in alcohol dependence. We think that, difference in population groups and selective inclusion criteria for alcohol dependence may affect results. Thus, we studied GABRG2 and ADH gene polymorphisms in Turkish population.

150 volunteers with no physical or psychological/mental diseases history and 100 patients who admitted to Ege University Alcohol Dependence Unit between years of 2005-2006 enrolled to the study. We found, significant increase in T allele and TT genotype in GABRG2 polymorphism in patients compared to healthy controls. Moreover, significant differences were also determined for ADH1 Arg allele and Arg/Arg genotype.

Compared to previously studies, we found more profound connection between alcohol dependence and GABRG2 gene polymorphism. Alcohol dependence is an important health problem depends many genetic and environmental factors but we think that it is possible to interpretation genetic risk for developing early diagnostic methods and treatment strategies by comprehensive linkage and association studies.

#### P08.31

##### The mathematical model of prediction of genetic targets in a limited amount of their measurements

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Pattern studies of genetic processes of the Russian populations and ethnic groups, is one of the problems of medical genetics. Genogeographic maps today have become well-designed chart patterns, reflecting genogeographic structure and epidemiology of hereditary diseases.

The specialists of the Medical Research Centre for Medical Genetics developed a mathematical model that provides a forecast incidence of a wide range of hereditary diseases within the studied population. As a basis for developing the model they employed the experimental results of population studies of Kirov, Kostroma, Bryansk, Tver, Rostov, Arkhangelsk, Krasnodar Region, the Republic of Mari El, Adygea, Chuvashia and Udmurtia. The software developed from the known analogues has the following major points:

- flexible structure with the possibility of automatic adaptation of the model prediction, depending on the number of settlements, where the population-genetic studies were carried out;
- the possibility of calculating the predicted prevalence of hereditary diseases for each municipality of the population under study, considering its geographical location and population number;
- nonequilibrium of the data, involved as "strong" values in the model prediction, taking into account the geographic and population proximity of the settlement, for which the forecast is performed, and the settlements, where the experimental population-genetic studies were conducted.

The developed model is implemented as an autonomous software system, with a simple interface that supports data input and storage, as well as visualization unit, providing automatic display of genogeographic maps reflecting the incidence of hereditary diseases within the population under study, based on the data entered by the user.

#### P08.32

##### Genome-wide linkage scan of human cognitive abilities in a large Dutch pedigree

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We performed a genome-wide multipoint linkage analysis of normal variation of cognitive function in an extended pedigree from the Erasmus Rucphen Family study. The study included 2883 subjects (mean age 48) characterized by eight cognitive tests. The test battery was developed to target endophenotypes for Alzheimer's disease (AD) but as expected for endophenotypes may also involve other disorders. Four composite scores for visuospatial ability, memory, executive function and global cognition were constructed. Pedigrees were split to the maximum bit-size of 18 and non-parametric linkage analysis was performed using MERLIN. Analysis was performed in the total population, and after stratification for age. Highest LOD scores were obtained in the analysis of persons <50 years old. In this group visuospatial ability demonstrated linkage to 1q (LOD=3.12) and 10q (LOD=2.39); memory to 11q, 12p, 12q and 14q (LODs between 1.96 and 2.53); executive function to 2q, 5q, 12q, 19q (LODs from 1.89 to 2.92) and global cognition linked to 9p and 12p (LODs of 2.97 and 1.88, respectively). Locus 12p was the same for memory and global cognition and locus 12q overlapped for executive function and global cognition. Our scan showed linkage of cognitive functions to known loci of AD: 19q (APOE region), 1q (PSEN2), 2q14 (IL1B), 9p (AD11 locus), 10q (PLAU), 11q (SORL1), 12p (OLR1), 12q (MAPT), and schizophrenia: 1q (PLXNA2), 11q (DRD2), 2p (GRIN2B), 12q (DAO). Overlap of linkage regions between the cognitive functions analyzed and Alzheimer's disease and schizophrenia prove that the cognitive abilities prove to be powerful endophenotypes.

#### P08.33

##### Public interest and expectations concerning commercial genotyping and genetic risk assessment

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Research Institute of Medical Genetics SB RAMS, Tomsk, Russian Federation. In the scientific world there is no current agreement about the utility of predictive genetic testing for multifactorial diseases. Nonetheless it is already being commercialized by a number of companies who provide direct-to-consumer genetic diagnostics.

We interrogated 2000 of Russian respondents about their desire to estimate their genetic risk for future diseases, their willingness to change lifestyles if the tests suggested a high risk of a disease, and a number of other related questions. We have revealed extremely high level of interest regarding new genetic developments and steady desire of people to learn more about their inheritance. Most people would agree to undergo genetic testing for common diseases. An absolute majority of respondents (89%) stated they will change their lifestyles in order to avoid a disease if there was a high risk. Our data confirms that lay people have highly overestimated expectations about the use of genetic technologies with respect to common diseases. Thus, 70% of the survey participants agreed with the statement that genetic tests should be extensively promoted and 81% agreed that knowing genetic mechanisms of diseases will help people to live longer and same proportion of respondents thinks that knowing their own genetic variants allows people to control their lifestyle easier.

The high level of public interest to personal genetic testing is promoting genetics commercialization. It is becoming clear that consumers of genetic diagnostics' services will define the pace of acceptance of genetic applications into medicine and other spheres of life.

**P08.34****Comprehensive approach to investigate the genetic basis of hereditary hearing loss in Iranian population**

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Genetic testing for deafness in Iran is well established. The population is extremely heterogeneous, which means that ethnic-specific data are required. We have generated much of these data by screening over 2000 families segregating autosomal recessive non-syndromic deafness (ARNSD). All patients were screened for mutations in *GJB2* and *GJB6* (DFNB1), and if no mutations were identified, haplotypes were reconstructed by typing three short tandem repeat polymorphisms flanking 22 known ARNSD loci. In a subset of families, genome-wide linkage analysis was completed. Our data show that 16.7% of the Iranian population with ARNSHL segregates mutations in *GJB2*. The most prevalent mutation in this gene was 35delG, although 34 different mutations have been identified of which seven are common. We have also identified a novel *GJB2* mutation in an endogenous population segregating ADNSHL in village in northern Iran. In approximately 30% of families, we have been able to establish a genetic cause for deafness. Over half have mutations in *GJB2*, and followed by mutations in *SLC26A4*, *TECTA* and *USH1C*. We have also found mutations in *PJVK*, *TMC1*, *USH1C*, *OTOF*, *MYO11A* and *VLGR1*. Lastly, we have described a new syndrome, a contiguous gene deletion syndrome that involves both deafness and infertility in males (DIM Syndrome). These data from the Iranian population attest to its diversity and contribute to the current body of knowledge regarding the deafness of genetics.

**P08.35****Evidence for genetic heterogeneity of bipolar disorder according to age at onset in 2q14**

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The aim of the present study is to perform a fine mapping of 8 regions previously identified by a genome-wide linkage analysis in European families ascertained through an early onset bipolar affective disorder (BPAD) type I proband (Etain et al., 2006). Early age at onset was defined as an age at first thymic episode below 21 years. The initial sample of 70 families was extended to 120. Probands were early onset bipolar type I patients and affected sibling were either early onset BPAD type I patients, later onset of BPAD type I or early onset BPAD type II. Since presence of genetic heterogeneity of BPAD may exist according to the phenotypic heterogeneity of siblings, The Predivided Sample Test and the Maximum Likelihood Binomial methods were used to test genetic heterogeneity using 138 independent affected sib-pairs. Of the 8 regions of linkage suggested by our previous genome scan, six regions (2q14, 3p14, 5q33, 7q36 16p23 and 20p12) remained linked to BPAD. Regions (2p21 and 10q23) were not confirmed. A genetic heterogeneity was revealed between early and late onset BPAD type I in the 2q14 region ( $p=0.0001$ ) using the two methods. For BPADI sib-pairs concordant for early age at onset, parental allele sharing proportion is higher (0.58) than for discordant pairs for age at onset, i.e. late onset BPADI (0.28). All these results show for the first time the underlying genetic heterogeneity in bipolar affective disorder and validate the age at onset as a relevant factor in its genetic vulnerability.

**P08.36****Frequency of single nucleotide polymorphisms in ABCG2 gene in Japanese hyperuricemic patients**

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Gout is a heterogeneous diseases resulting from tissue deposition of uric acid crystals, based on hyperuricemia. Uric acid is the end product of human purine metabolism and serum uric acid concentration

is determined by the balance between production and elimination. The kidney plays a main role in uric acid elimination. The transporters for uric acid have been identified such as URAT1, OAT1, OAT3 and MRP4. Several recent genome-wide association studies identified associations between single nucleotide polymorphisms (SNPs) in the other transporter genes, *SLC2A9*, *SLC17A3* and *ABCG2*, and serum uric acid concentration and gout. For evaluation of influence of *ABCG2* on serum uric acid concentration, we identified the frequency of SNPs in *ABCG2* gene of 198 Japanese hyperuricemic patients. As a result, the frequency of some SNPs has been higher than that of Japanese population. It suggests that the functional variation of *ABCG2* due to the SNPs influences serum uric acid concentration.

**P08.37****Allele frequency of two autosomal STR loci FGA and D7S820 in Iranian population**

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Short tandem repeat (STR) genetic loci were assigned for use in forensic investigation. Allele frequency of two autosomal STR loci FGA and D7S820 were determined in Iranian population.

DNA from 579 or 556 unrelated individuals were analyzed for D7S820 and FGA markers respectively. Extracted DNA were amplified by using AmpF STR Identifier Kit and 3130 Genetic Analyzer from ABI (US). Allele frequencies and forensic efficiency parameters such as Heterozygosity (H), Polymorphism Information Content (PIC), Power of Discrimination (PD), Propability of Match (PM), Power of Exclusion (PE), and typical Paternity Index (PI), were calculated using the PowerstatsV12.xls software (Promega, US).

A total of 11 alleles for D7S820 and 13 alleles and 9 interalleles for FGA could be observed. In the case of FGA, allele 23 showed the highest frequency (18.2%) and alleles 16, 28 and interalleles 18.2, 31.2 had the lowest frequencies (0.1%). Allele range in FGA was from 16 to 31.2 repeats with lower match propability (MP=0.034) compared with D7S820 (MP=0.066). In D7S820 marker the highest frequency (25.9%) was belonged to allele 11 and the lowest frequencies (0.1%) were found to be for repeats 29, 30. Also the highest PD (0.966) and PE (0.658) belonged to FGA. We also observed several interalleles for FGA marker. But there was not any evidence of interalleles for D7S820 locus. The results indicated that the both loci were in Hardy-Weinberg equilibrium.

**P08.38****A genetic study of the VWA and THO1 short tandem repeat systems in Iranian population**

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Several STR markers have been investigated to be routinely used for forensic purposes. Allele frequencies of the two short tandem repeats (STRs) namely VWA and THO1 were determined in Iranian population. DNA from 573 or 596 unrelated individuals were analyzed for THO1 and VWA markers respectively. Extracted DNA were amplified by using AmpF STR Identifier Kit and 3130 Genetic Analyzer from ABI (US). Allele frequencies and forensic efficiency parameters such as Heterozygosity (H), Polymorphism Information Content (PIC), Power of Discrimination (PD), Propability of Match (PM), Power of Exclusion (PE), and typical Paternity Index (PI), were calculated using the PowerstatsV12.xls software (Promega, US).

A total of 16 alleles for VWA and 16 alleles for THO1 could be observed. In the case of VWA, allele 17 showed the highest frequency and alleles 8, 9, 9.3, 10, 11, 12, 15.2 had the lowest frequencies. Allele range was from 8 to 20 repeats with higher match propability (MP=0.083) compared with THO1.

In THO1 marker the highest frequency was belonged to allele 6 and the lowest frequencies were found to be for repeats 5, 13, 17. Also the highest PD and PE belonged to THO1. We also observed several interalleles for both markers. In this study both loci provided allelic frequencies of high heterogeneity to use them for forensic and related purposes.

**P08.39****Leptin and leptin receptor gene variation and human obesity**

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Leptin is a protein hormone with important role in regulation of body weight, metabolism, reproductive function, modulation of immune response and angiogenesis. As a regulator of body weight, it operates by inhibiting food intake and stimulating energy expenditure and its concentration in the blood serum is proportional to the amount of body fat. Polymorphic leptin and leptin receptor genes have often been investigated as possible factors associated with human obesity. We tested polymorphisms G-2548A in the promoter region and A19G in exon 1 of the leptin gene and polymorphisms G-1041A in the promoter region, Arg109Lys in exon 4 and Arg223Gln in exon 6 of the leptin receptor gene for association with leptin concentration and obesity. Allelic frequencies of these polymorphisms show inter-poplational variation. A population-based association study was conducted in the population isolate of the Eastern Adriatic island of Vis, Croatia (N=243, age 22-85 yrs). Obesity was defined as  $BMI \geq 30 \text{ kg/m}^2$ . Leptin concentration was significantly higher in the obese (65.3 ng/ml), than in the non-obese (19.9 ng/ml) and in women (44.6 ng/ml) compared to men (13.8 ng/ml). The results indicated significant association of -2548G variant and leptin concentration in men ( $p=0.013$ ). No association of the other studied polymorphisms with leptin concentration was found, and none of the studied polymorphisms showed association with obesity (with leptin concentration, sex and age as covariates). The lack of association could be due to the complex pathogenesis of obesity, which involves a number of genetic and environmental factors.

**P08.40****Statistical properties of tests of association performed on mixtures of singletons and related individuals: effects of the nonorthogonality of linkage and LD parameters on type I error and power**

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The current trend in mapping of the complex diseases is genome-wide association by analyzing anonymous SNP markers in cohorts of unrelated cases and controls. A motivation for this is that unrelated individuals sharing some phenotype are much easier to collect than large families with multiple affected persons, when the genetic portion of the phenotypic etiology is incomplete.

In this study, we examined the statistical properties of several commonly used family-based association tests in genetic epidemiology as to their performance using real-life mixtures of families and singletons taken from our own migraine and schizophrenia studies. We simulated a disease conditional on the known phenotype structures in these pedigrees under a variety of inheritance models in which one variant in a given gene region influences the trait to some degree.

The results of our study showed the in virtually every situation, the full likelihood-based methods outperformed the simpler "data structure-motivated" tests. In truth we never know the true analysis models, so we noticed that the power of a joint-test of linkage and association was robust to model errors (so long as the analysis model is overly determined), the test of association conditional on linkage can have difficulty parsing the signal from LD and linkage satisfactorily under certain analysis options, owing to the nonorthogonality of those parameters. A simulation-based as well as formal analytical description and explanation will be presented, along with discussions of bias-correction methods to restore the validity of this family of powerful and highly sensitive tests.

**P08.41****Analytic approaches to power estimation for linkage analysis of large pedigrees**

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The variance-components method is widely used for linkage mapping of quantitative trait loci. Estimation of power for this method is based on asymptotic approximation of distribution of likelihood ratio statistics to a non-central chi-squared distribution described by a non-centrality parameter (NCP). Therefore, evaluating the power can be reduced to an estimation of the NCP. For small pedigrees, the NCP can be analytically expressed by simple formulas. For large pedigrees, analytical formulas cannot be deduced because the expectation of the test statistics must be estimated for all possible genotype vectors, the number of whose grows exponentially with increasing pedigree size. Recently Williams and Blangero (1999) and Rijssdijk et al. (2001) have suggested ways of approximating the NCP by the sum of NCP values for all pairs of relatives. Expectation of the NCP for any related pair may be calculated analytically. The effectiveness of this approach was demonstrated for small pedigrees. We have investigated using this approach for analysis of large pedigrees. We have compared two ways of the analytical NCP approximation showing their equivalence, and investigated the accuracy of the analytical NCP estimation for three large pedigrees and for wide set of models of quantitative trait inheritance. We have demonstrated that sample size estimated by the NCP approximation was slightly overstated (up to 8 %) as compared with sample size calculated through the exact NCP value. This overestimation was the same for large and small pedigrees. So, a special correction could be introduced to obtain unbiased estimation of the NCP.

**P08.42****Evidence for a susceptibility locus for Ménière's disease on chromosome 12p12.3.**

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Ménière's disease (MD) is a disorder of the inner ear characterized by episodes of vertigo, tinnitus and fluctuating sensorineural hearing loss. Most MD cases are sporadic but 5-15% of patients are familial following an autosomal dominant mode of inheritance with incomplete penetrance. The genetic cause of the disease remains unknown. We have previously identified a candidate region for MD on 12p12.3 from a linkage analysis in three large Swedish pedigrees. Interestingly, affected individuals of two families share a single haplotype within the linked region, suggesting a possible ancestral mutation.

To further clarify the role of chromosome 12p in MD we genotyped 15 Swedish families with familial cases of the disease. We analyzed 11 polymorphic marker loci over a 2Mb region in samples from affected individuals and healthy control subjects. The results revealed association of 5 polymorphic marker alleles to MD ( $P < 0.05$ ). Moreover, a core haplotype spanning 700 kb showed strong association with the disease ( $P = 0.0041$ ). In conclusion, our results indicate a susceptibility locus for familial MD in the 12p12.3 region.

**P08.43****Matrix metalloproteinase 9 -1562 C/T gene polymorphism and susceptibility to abdominal aortic aneurysm or aortoiliac occlusive disease**

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Objective: Abdominal aortic aneurysms (AAA) and aortoiliac occlusive disease (AIOD) are the most frequent reasons for vascular surgery procedures within abdominal cavity. Genetic factors responsible for individual risk of development of these diseases are little known. Both AAA and AIOD are considered to have multifactorial etiology. Although these diseases are distinct pathological processes however they have common risk factors and location. Involvement of various matrix metal-

loproteinases is considered to be very important in the pathogenesis of these diseases. Matrix metalloproteinase 9 (MMP-9) was shown to be implicated in the pathogenesis of AAA and functional polymorphism in the promoter region (-1562 C/T) of the MMP-9 gene was reported to be associated with AAA in single report. The purpose of the present study was to determine if there is an association between the MMP9 -1562 C/T genotype and susceptibility to abdominal aortic aneurysm or aortoiliac occlusive disease in Polish patients.

**Methods:** Based on the PCR-RFLP analysis MMP9 genotypes were determined in three selected groups: 182 patients with AAA and 205 patients with AIOD who underwent surgery; 200 healthy individuals from control group. Genotypes were compared with demographic and clinical data of subjects and analyzed in relation to risk factors.

**Results:** There were no significant differences in MMP9 -1562 C/T genotype frequencies between AAA patients, AIOD patients and control subjects.

**Conclusion:** No association of MMP9 -1562 C/T gene polymorphism with abdominal aortic aneurysm or aortoiliac occlusive disease was found.

#### P08.44

#### No association between myeloperoxidase G-463A polymorphism and rheumatoid arthritis in Turkish Patients

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Myeloperoxidase (MPO) has been involved in the pathogenesis of several diseases such as Rheumatoid Arthritis (RA) through excessive production of reactive oxygen species (ROS) as well as through its genetic polymorphism. We examined whether G-463A polymorphism of Myeloperoxidase (MPO) gene was associated with RA.

Exactly, 75 patients with RA and 150 healthy control subjects were included in this study. The genotyping was determined by polymerase chain reaction-restriction fragment length polymorphism method. The association between these SNP and RA was analyzed using chi-square test and de-Finetti program.

MPOG-463A genotype distributions and allele frequency of RA patients were not significantly different from healthy controls. In addition, it was also determined that there was no deviation from Hardy-Weinberg Equilibrium in any groups ( $p>0.05$ ).

Whether there was an association between MPOG-463A gene polymorphism and RA was investigated for the first time in this study in literature and it was demonstrated that it did not exist in the Turkish RA patients. It was planned to investigate the other polymorphisms of MPO gene in the future.

#### P08.45

#### Folate pathway gene polymorphisms and development of myopathy process in PMD patients (Moldavian population)

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#### Background:

During literature analysis we have found hypothesis that frequency of MTHFR C677T mutation in its heterozygous state had a tendency to increase due to its positive effect on some diseases, that lead to increased survival of carriers. We set up that in Moldavian population, PMD patients with different clinical features and severity of pathology process. Main goal of present study was to determine if there is an interaction between MTHFR polymorphism and development of myopathy process.

#### Materials and methods:

110 subjects were genotyped for the MTHFR 677 variants, among them 55 patients with PMD and 55 age-matched healthy control subjects. Blood samples were collected and DNA was isolated from peripheral blood leukocytes. MTHFR variant alleles were determined by a PCR-RFLP. Detection of the MTHFR C677T was performed according original protocol with primers from Alpha DNA. Statistic analyze was performed in SISA program.

#### Results:

We have found that MTHFR C677C allele presents among 38 (69.1%) PMD and 22 (40.0%) controls ( $X^2=9.39$ ;  $p=0.002$ ), the C677T genotype among 11 (20.0%) PMD, 24 (43.6%) controls ( $X^2=7.08$ ;  $p=0.007$ ), and the T677T allelic variant was observed 6 (10.9%) PMD, 9 (16.4 %)

controls ( $X^2=0.695$ ;  $p=0.405$ ).

#### Conclusions:

Mathematic analyze of obtained data demonstrates statistically significant differences between PMD and control MTHFR genotypes. Frequency of MTHFR C677T was higher in control group. Whereas in PMD patients more often MTHFR C677C allelic variant. This study needs to be continued with MTHFR A1298C, MTRR A66G and MTR A2756G allelic variants for learning compound effect.

#### P08.46

#### Genetic heterogeneity of Multiple Self-Healing Squamous Epithelioma in a Tunisian family not linked to chromosome 9 (9q22.3-q31)

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Multiple self-healing squamous epithelioma (MSSE), also known as Ferguson-Smith Disease, is a rare genodermatosis with an autosomal dominant inheritance. Affected patients suffer from recurrent skin lesions, which clinically and histologically resemble to keratoacanthomas or well-differentiated squamous cell carcinomas, but with a spontaneous regression, leaving only atrophic scars. This disease has been linked to 9q22.3-31 locus between D9S197 and D9S1809 markers. This region has a size of 4 cM (2.05 Mb).

Here we investigate a five generation Tunisian family in which 7 members are affected by MSSE. A detailed disease history with particular attention to the age of onset, distribution, and clinical course of their skin lesions were noted and a full clinical examination was performed for every patient. Haplotype analysis, using six polymorphic short tandem repeat markers in 9q22.3-31 locus, doesn't find any commune haplotype segregating with MSSE in this family. So that proposes the existence of at least one other locus linked with this disease and suggests its genetic heterogeneity feature.

#### P08.47

#### A novel syndromic neuro-ichthyosis maps to chromosome Xq22-24

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The term neuro-ichthyosis refers to a clinically and genetically heterogeneous group of syndromes in which ichthyosis can be found in association with various neurological disorders, spanning from mental retardation to spasticity, epilepsy, polyneuritis and pyramidal tract signs. These syndromes are very rare; despite of the fact that they could cluster into families, many still lack a locus assignment. For some of the forms for which a locus has been recognized, the disease gene has been identified. The mode of inheritance could be either autosomal or X-linked, dominant or recessive. Generally, X-linked ichthyosis is due to steroid sulfatase deficiency, which is determined by deletions of the STS gene on chromosome Xp22. Here, genetic mapping of a novel form of X-linked ichthyosis undue to STS deficiency is reported. We performed an X chromosome linkage analysis with 31 markers on 16 DNA samples. A clinical picture of ichthyosis, agenesis of corpus callosum (ACC), microcephalia, seizures, mental retardation and spastic tetraparesis characterized the proband and his 1st degree cousin died at age 6. Intriguingly, mother of this latter child interrupted a second pregnancy in the second trimester, and phoetus showed clear hyperkeratosis and complete ACC. The two affected males and the fetus inherited the same haplotype on chromosome Xq22-24; multipoint linkage analysis yielded LOD scores of 2.06 in this region, containing 230 genes. An array-CGH scan on the proband at a 20 kb resolution demonstrated that no copy number variations occurred. Mutational screening of the Xq22-24 genes expressed in skin is in progress.

**P08.48****Genetic polymorphism in the matrix metalloproteinases genes and risk of occupational chronic bronchitis**O. Tselousova<sup>1</sup>, G. Korytyna<sup>1</sup>, L. Akhmadishina<sup>1</sup>, T. Victorova<sup>1,2</sup>;<sup>1</sup>Institute of Biochemistry and Genetics, Ufa, Russian Federation, <sup>2</sup>Bashkir State Medical University, Ufa, Russian Federation.

Active occupational exposure to respiratory irritants is the major risk factors for occupational chronic bronchitis (OCB), but OCB develops in some workers. It's suggested a significant genetic role. Matrix metalloproteinases (MMPs) are proteolytic enzymes associated with inflammation and airway remodelling in respiratory diseases. The aim of this study was to investigate the role of MMPs polymorphisms in the development of occupational chronic bronchitis in workers from Russia. The MMP1 (interstitial collagenase), MMP9 (gelatinase B) and MMP12 (macrophage elastase) were genotyped by PCR-RFLP analysis in 110 workers with OCB and 157 healthy workers.

The overall, the genotype distribution and the allele frequencies of polymorphisms G(-1607)GG of MMP1 gene, A(-82)G of MMP12 gene and C(-1562)T of MMP9 gene didn't significantly differ in groups (all P values are above 0.05). The most common MMP1 genotype was GG/GG in the patients with OCB (60.26%). The MMP9 frequency distribution CC genotype was similar in the workers with OCB and in the healthy subjects (80.91% and 73.95%, respectively). The frequency of MMP12 AA genotype between the workers with OCB (75.73%) and the healthy workers (72.61%) was not significant.

Our results show no significant difference between the workers with occupational chronic bronchitis and the healthy workers for MMPs. It's needed more studies to define genetic risk factors for occupational chronic bronchitis.

**P08.49****Genome-wide parametric linkage analysis of adult height**T. I. Axenovich<sup>1,2</sup>, I. V. Zorkoltseva<sup>1</sup>, N. M. Belonogova<sup>1,2</sup>, A. V. Kirichenko<sup>1</sup>, B. A. Oostra<sup>3</sup>, C. M. van Duijn<sup>3</sup>, Y. S. Aulchenko<sup>1,3</sup>;<sup>1</sup>Institute of Cytology and Genetics, Novosibirsk, Russian Federation, <sup>2</sup>Novosibirsk State University, Novosibirsk, Russian Federation, <sup>3</sup>Erasmus MC Rotterdam, Rotterdam, The Netherlands.

Despite extensive research of genetic determinants of human adult height, the current knowledge allows us to predict only a small portion of the trait's genetic variation. In this study we analyzed 2940 genotyped and phenotyped individuals in a large pedigree including more than 23000 members in 18 generations. The pedigree was derived from an isolated Dutch population, where genetic heterogeneity is expected to be low and linkage disequilibrium to be extensive.

Complex segregation analysis confirmed high heritability of adult height, and suggested involvement of a major gene in the control of height in this population. The estimates obtained from complex segregation analysis were used to perform parametric linkage analysis. Parametric linkage analysis indicated at least four suggestive and three genome-wide significant loci. Significant peaks were located at the chromosome regions 1p32.2 (LOD score = 3.35), 2p16.3-21 (LOD score = 3.29) and 16q24.1-24.2 (LOD score = 3.94). First peak was close to already known candidate gene COL9A2. The second peak was in the middle of neurexin 1 gene (NRXN1), which is expressed in hypothalamic area and may influence growth hormone production. The locus 16q24.1-24.2 showing the strongest linkage signal was not mapped earlier as contributing to population diversity of human stature.

Despite of relatively small number of genotyped and phenotyped individuals a genome-wide parametric linkage identified four loci with suggestive linkage and three with significant linkage. This demonstrates high power of the used approach, which combines advantages of genetically isolated population, large pedigree and parametric methods of linkage analysis.

**P08.50****A 3'UTR transition within DEFB1 is associated with chronic and aggressive periodontitis**G. M. Richter<sup>1</sup>, A. S. Schäfer<sup>1</sup>, M. Nothnagel<sup>2</sup>, M. L. Laine<sup>3</sup>, A. Röhling<sup>4</sup>, C. Schäfer<sup>5</sup>, N. Cordes<sup>4</sup>, B. Noack<sup>5</sup>, M. Folwaczny<sup>7</sup>, J. Glas<sup>7</sup>, C. Dörfer<sup>4</sup>, H. Domisch<sup>5</sup>, B. Groessner-Schreiber<sup>4</sup>, S. Jepsen<sup>5</sup>, B. G. Loos<sup>8</sup>, S. Schreiber<sup>1</sup>;<sup>1</sup>Institute for Clinical Molecular Biology, University Medical Center Schleswig-Holstein, Kiel, Germany, <sup>2</sup>Institute of Medical Informatics and Statistics, Uni-

versity Medical Center Schleswig-Holstein, Kiel, Germany, <sup>3</sup>Academic Center for Dentistry, VU, Amsterdam, The Netherlands, <sup>4</sup>Department of Operative Dentistry and Periodontology, University Medical Center Schleswig-Holstein, Kiel, Germany, <sup>5</sup>Department of Periodontology, Operative and Preventive Dentistry, Bonn, Germany, <sup>6</sup>University Medical Center Carl Gustav Carus, Dresden, Germany, <sup>7</sup>Department of Preventive Dentistry and Periodontology, Munich, Germany, <sup>8</sup>Department of Periodontology, Academic Centre for Dentistry Amsterdam (ACTA), VU, Amsterdam, The Netherlands.

Periodontal diseases are complex inflammatory diseases and affect up to 20% of the worldwide population. An unbalanced reaction of the immune system to microbial pathogens is considered the key factor in the development of periodontitis. Defensins have a strong antimicrobial function and are important contributors of the immune system in maintaining health. We present the first systematic association study of DEFB1. Using a tagging SNP approach including described promoter SNPs of DEFB1, we investigated the associations of the selected variants in a large population (N = 1,337 cases and 2,887 ethnically matched controls). The 3'UTR SNP rs1047031 showed the most significant association signal for homozygous carriers of the rare A allele (P = 0.002) with an increased genetic risk of 1.3 (95% confidence interval 1.11-1.57). The association was consistent with the specific periodontitis forms chronic periodontitis (odd's ratio = 2.2 [95% confidence interval 1.16-4.35], P = 0.02), and aggressive periodontitis (odd's ratio = 1.3 [95% confidence interval 1.04-1.68], P = 0.02). Sequencing of regulatory and exonic regions of DEFB1 identified no other associated variant, pointing to rs1047031 as likely being the causative variant. Prediction for microRNA targets identified a potential microRNA binding site at the position of rs1047031.

**P08.51****Pharmacogenetic investigation in complex traits using Genome Wide Association Study**E. Salvi<sup>1</sup>, C. Barlassina<sup>1</sup>, L. Citterio<sup>2</sup>, C. Lanzani<sup>2</sup>, S. Lupoli<sup>3</sup>, F. Torri<sup>4</sup>, A. Orro<sup>4</sup>, C. Cosentino<sup>1</sup>, F. Taddeo<sup>1</sup>, V. Tieran<sup>1</sup>, D. Cusi<sup>5</sup>, G. Bianchi<sup>2</sup>, F. Macciardi<sup>1</sup>;<sup>1</sup>University of Milan, Milan, Italy, <sup>2</sup>University Vita Salute San Raffaele, Milan, Italy, <sup>3</sup>INSPE, HSR Scientific Institute, Milan, Italy, <sup>4</sup>Institute of Biomedical Technologies, CNR, Segrate, Milan, Italy, <sup>5</sup>San Carlo Borromeo Hospital, Milan, Italy. Pharmacogenetic association studies help to identify DNA variants which impact on the individual response to drugs. The knowledge of sample variability in drug response can allow to personalize drug dosing and treatment regimes. The fundamental question concerns whether it is possible to differentiate the patients with potentially responses to the treatment (R) from those with the greatest risk of no response (NR). Our approach to these questions is to identify SNPs that segregate with drug efficacy either in candidate genes (CG) that relate to the mechanism of action of the drug or in other DNA regions detected with a Genome Wide Association Study (GWAS). SNPs identified from CG or GWAS could allow NR to be excluded from subsequent clinical trial studies, therefore allowing enriched, smaller, faster, less expensive clinical studies on patients with a better chance of responding favorably. Here, we present an example of this approach: we adopted a genetic association design, where the phenotype of interest was measured as a quantitative trait and the variables affecting the distribution of the phenotype are the SNPs, the therapy and the SNP\*therapy interaction. Once we identified those genes, we developed an algorithm to detect the genotypic profiles that best discriminate R from the NR. In a final step, we merged the best predictive SNPs found from our GWAS strategy with those from a CG approach. Both categories of SNPs convey a specific predictive power that is magnified by their joint integration into a unified model.
**P08.52****The hereditary risk factors of socially significant multifactorial diseases**O. A. Gra<sup>1,2</sup>, Z. M. Kozhekaeva<sup>2,3</sup>, D. V. Gra<sup>4</sup>, M. D. Fedorova<sup>4</sup>, O. I. Skotnikova<sup>5</sup>, N. P. Kisseljova<sup>4</sup>, F. L. Kisseljov<sup>4</sup>, I. V. Goldenkova-Pavlova<sup>2</sup>, T. V. Nasedkina<sup>1</sup>;<sup>1</sup>Engelhardt Institute of Molecular Biology Russian Academy of Sciences, Moscow, Russian Federation, <sup>2</sup>Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, Russian Federation, <sup>3</sup>Institute for Human Genetics, University of Miami Miller School of Medicine, Miami, FL, United States,<sup>4</sup>Institute of Carcinogenesis, N. N. Blokhin Cancer Research Centre, Russian Academy of Medical Science, Moscow, Russian Federation, <sup>5</sup>The Central Sci-

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The predictive diagnostics of socially significant diseases with a multifactorial etiology becomes more significant due to worsening of ecological conditions and increasing of the number of chronic pathologies.

We developed Pharmagen-biochip for the polymorphism analysis in the candidate genes controlling the biotransformation system and the NAT2-biochip for the analysis of all significant variants in NAT2 gene. Our analysis has revealed genetic risk factors for development of several multifactorial pathologies: childhood acute leukemia (polymorphic variants of genes GSTT1, GSTM1, NAT2 and MTRR), leukemia and lymphoma in adults (polymorphic variants of genes CYP1A1, GSTM1 and CYP2C9), and the lung diseases in children and adults (polymorphic variants of genes CYP2C9, GSTT1, GSTM1 and NAT2). The analysis of genes predisposing to cervical cancer has defined risk factors of oncopathology development (polymorphic variants of genes CYP1A1, GSTM1, MTHFR and NAT2). In addition, it has been showed that polymorphic variants of CYP1A1, GSTT1, GSTM1 and NAT2 genes may consider as predictive markers for risk of relapse in childhood acute leukemia and could be used to individualize the standard therapy.

Our results show the importance of the genetic risk factors analysis in multifactorial diseases and the utility of biochips as genotyping instrument in preclinical diagnostics and personalized medicine for individual adjustment of drug dosage in clinic.

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#### P08.53

##### Recurrent oncostatin M receptor gene mutations and haplotype association in primary cutaneous amyloidosis in Taiwan

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Primary cutaneous amyloidosis (PCA) is characterized by severe itching, maculopapular lesions, pigmentation, and amyloid deposits in the dermal papilla. The disease is relatively common in Southeast Asia and South America. Previously, we reported familial clustering and genetic heterogeneity of PCA and mapped the condition to 5p13.1-q11.2 in a subset of pedigrees from Taiwan. A gene within this region, OSMR, has recently been found to harbor mutations in familial cases. Here, we investigated 29 PCA pedigrees and found that 10 had heterozygous missense mutations in OSMR, all of which occurred within the fibronectin type III-like repeat domains of the encoded OSMR $\beta$  protein: p.D647V (1 family), p.P694L (6 families), and p.K697T (3 families). The mutation p.P694L was associated with the same haplotype in 5 of 6 families. This particular mutation and haplotype were also detected in 2 sporadic cases of PCA, suggesting a common ancestral origin. No mutations in OSMR were identified in the other 19 pedigrees with familial PCA or in 89 sporadic cases of PCA, findings consistent with other disease-associated genes or perhaps non-genetic causes in some individuals. Our study provides insight about the complex genetic etiology of PCA and shed light on cytokine receptors and PCA pathogenesis.

#### P08.54

##### Functional variant of the PTPN22 gene associated with Graves' disease predisposition in Slovenian population

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Recent findings have demonstrated that the single nucleotide polymorphism 1858C>T in the PTPN22 (protein tyrosine phosphatase nonreceptor 22) gene has functional relevance and is associated with a variety of autoimmune diseases. The aim of this study was to assess the role of the PTPN22 1858C>T polymorphism in the genetic predisposition to Graves' disease (GD) in Slovenian population. We analyzed a case-control cohort composed by 100 patients with Graves' disease and 100 healthy controls. The PTPN22 1858C>T genotyping was performed by TaqMan 5' allelic discrimination assay. The allele distributions followed the Hardy-Weinberg equilibrium. CC genotype was found in 71 patients and 88 controls, CT genotype in 27 patients and 11 controls, and TT genotype in 2 patients and 1 control. Results were further analyzed by Fisher's exact test. Allele and genotype frequencies of the PTPN22 1858C>T polymorphism between patients and controls were compared. Significantly different allele and genotype distribution was observed. The p-value for GD susceptibility was 0.0046 for patients carrying T allele (CT+TT) compared to controls. As allelic variation of 1858C>T polymorphism could alter T - cell signalization we believe that PTPN22 gene holds a disease-predisposing allele that contributes to development of GD. Therefore, our data suggest that the PTPN22 1858C>T single nucleotide polymorphism has an effect on GD susceptibility in Slovenian population.

#### P08.55

##### Molecular diagnosis of known recessive ataxias by homozygosity mapping with SNP arrays

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**M. Anheim**<sup>1</sup>, **L. Ali-Pacha**<sup>5</sup>, **C. Lagier-Tourenne**<sup>1</sup>, **N. Drouot**<sup>1</sup>, **C. Thibaut**<sup>1</sup>, **T. Benhassine**<sup>6</sup>, **Y. Sif**<sup>2</sup>, **J. Poujet**<sup>7</sup>, **A. Hamri**<sup>2</sup>, **F. Hentati**<sup>3</sup>, **R. Amouri**<sup>3</sup>, **F. Santorelli**<sup>4</sup>, **M. Tazir**<sup>6</sup>, **M. Koenig**<sup>1</sup>;

<sup>1</sup>IGBMC, Strasbourg, France, <sup>2</sup>Centre Hospitalo-Universitaire Ben Badis, Constantine, Algeria, <sup>3</sup>Institut de Neurologie, Tunis, Tunisia, <sup>4</sup>Molecular Medicine & Dept. of Neurosciences IRCCS Ospedale Bambino Gesù, Roma, Italy, <sup>5</sup>Service de Neurologie, Centre Hospitalo-Universitaire Mustapha, Alger, Algeria, <sup>6</sup>Institut Pasteur, Alger, Algeria, <sup>7</sup>Hopitaux Universitaires de Marseille, Marseille, France. The diagnosis of rare inherited diseases is becoming increasingly difficult as an increasing number of affections appears to have multigenic inheritance. Multigenic inheritance applies for the recessive progressive ataxias, for which 12 genes have been identified. We used homozygosity mapping of patients with recessive ataxia and born from consanguineous parents, as a guide for identification of the defective locus. Patients from 97 families were analysed with GeneChip Mapping 10K or 50K SNP Affymetrix microarrays. We identified six families homozygous for regions containing the ARSACS gene, two families homozygous for the ataxia-telangiectasia gene, two families homozygous for the AOA1 gene, and one family homozygous for the AOA2 gene. A mutation was identified in all families homozygous for the ARSACS, AOA1 or AOA2 loci, and in only one of the two families homozygous for the A-T locus. The family without a mutation in the ATM gene was not reminiscent of A-T, as alpha-fetoprotein was normal in all 4 patients who had onset of ataxia at 8 years and absent reflexes. However, the LOD score in favor of linkage for the 11q22.1-q23.1 region was 3.2 and no other region of the genome was consistent with linkage for this family, suggesting that a second ataxia gene is present in this interval. While the use of homozygosity mapping was very effective at pointing to the correct ataxia gene, it also suggests that the majority of recessive ataxia cases are caused by mutations either in the recently identified genes or in genes yet to be identified.

#### P08.56

##### Pharmacogenomic study of drug transporters CYP3A4, CYP3A5 and ABCB1 on cyclosporine A and tacrolimus with allograft rejection in renal transplant recipients of north India

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Background: Calcineurin inhibitors cyclosporine (CsA) and tacrolimus (Tac), substrates of cytochrome P-450 3A (CYP3A) subfamily and ATP

binding cassette subfamily B member 1 (ABCB1) are associated with wide inter-individual heterogeneity in oral bioavailability. We therefore, investigated pharmacogenomic associations in *CYP3A4*, *CYP3A5* and *ABCB1* genes with allograft outcome in a cohort of 297 renal transplant recipients.

Methods: 224 patients on CsA and 73 patients on Tac based immunosuppression regimen were genotyped for single nucleotide polymorphisms (SNPs) in *CYP3A5* (*CYP3A5\*2*, *CYP3A5\*3*, *CYP3A5\*4*), *CYP3A4* (*CYP3A4\*1B*, *CYP3A4\*6*, *CYP3A4\*18*) and *ABCB1* (-129T>C, 1236C>T, 2064-76T>A, 2677G>T, 3435C>T) and correlated with CsA/Tac dose requirement (mg/kg/day) and dose adjusted CsA ( $C_2$ )/Tac ( $T_0$ ) blood levels (concentration/dose ratio) at 1month and 3months post transplantation.

Results: Daily dose requirements were 10-22% and 20-40% higher for CsA and Tac, respectively, in *CYP3A5\*3* \*1/\*1 genotype patients as compared to \*3/\*3 patients at 1 and 3 months post transplantation. Significant correlation of *CYP3A5\*3* with dose adjusted levels of CsA ( $r^2=0.047$ ,  $p=0.001$ ) and Tac ( $r^2=0.278$ ,  $p<0.001$ ) were observed at 1 and 3months. The dose-adjusted levels were also lower in *ABCB1* c.2677G>T GG genotype (CsA,  $C_2$   $p=0.032$ ; Tac  $T_0$ ,  $p=0.001$ ) suggesting that for a given dose their CsA/Tac blood concentration was lower. The GG genotype was further associated with lower allograft survival as indicated by Kaplan-Meier analysis ( $p=0.007$ ).

Conclusion: Identification of *CYP3A5\*3* \*1/\*1 and *ABCB1* 2677GG patients with significantly lower dose-adjusted concentrations suggest the requirement of higher CsA/Tac dose to reach therapeutic concentrations. The study may supplement pre-transplant pharmacogenomic information for individualizing immunosuppressant doses.

#### P08.57

#### Association study of HLA-DRB1 alleles with Rheumatoid Arthritis in Tunisian patients

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Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by progressive joint destruction and autoantibody formation. Auto-antibodies elaborated include anti-citrullinated protein/peptide antibodies (ACPA) and anti-rheumatoid factor (RF). Both genetic and environmental factors, and their interaction, play a role in the development of RA. A common set of alleles at the HLA-DRB1 locus (the shared epitope alleles) has been associated with RA in populations with white European and Asian ancestry. The aim of this study is to investigate the involvement of HLA-DRB1 alleles in the susceptibility to RA in the Tunisian population. A set of 143 RA patients and 123 Tunisian controls was analyzed for HLA-DRB1 alleles using polymerase chain reaction/sequence specific primers (PCR/SSP). Association was assessed based on the  $\chi^2$  test and odds ratios (ORs) with 95% confidence intervals (CIs). Our results showed a higher frequency but non significant association of HLA-DRB1\*04 and HLA-DRB1\*10 alleles in patients compared to the controls (23.43% versus 16.26%, 6.64% versus 2.44%; respectively). However, the stratification of our patients according to immunological and clinical data showed a significant difference of HLA-DRB1\*04 allele in the subgroup of patients with ACPA ( $p_c = 0.023$ ; OR= 2.08; CI= [1.31-3.3]) and the subgroup of patients with RF ( $p_c = 0.023$ ; OR= 2.09; CI= [1.31-3.34]). Moreover, the subdivision of patients according to sex, extra-articular involvement and radiographic damage showed no significant difference ( $p>0.05$ ). Our results indicated that the HLA-DRB1\*04 allele was significantly associated with RA susceptibility in both subgroups with ACPA and RF.

#### P08.58

#### Comparative analysis of the first trimester serum markers' levels in twin and singleton pregnancies

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Objectives: to look at the differences in the serum markers' level between singleton and multiple pregnancies.

Methods: in total 22 women in the age of 24 to 41 years old with twin pregnancy were recruited in the study group. Control group consisted

of 22 women with spontaneous singleton pregnancy. Serum markers (PAPP-A and free  $\beta$ -HCG) and fetal nuchal translucency (NT) thickness were measured at 10+0 to 13+0 weeks of pregnancy. All the women with twins underwent chorionbiopsy due to increased NT thickness or increased maternal age.

Results: Differences in the median of PAPP-A were significantly lower in singletons (1.09; 0.27 - 2.48) vs multiple pregnancies (2.69; 0.76 - 6.19),  $p<0.0001$  whereas the difference in the level of free  $\beta$ -HCG in singleton pregnancies were statistically not significant (1.08; 0.24 - 5.32) compared to twins (1.87; 0.64 - 5.16),  $p<0.132$ . Cytogenetic analysis of the 44 embryos had revealed 2 cases of aneuploidies - trisomy 21, trisomy 18. Both mothers were under the age of 35 years. The elective reduction of the affected embryos was performed without complications.

Conclusions: Twin pregnancies have shown a significantly higher level of PAPP-A compared to singleton pregnancies. A further research is needed to investigate the diagnostic efficiency of currently used serum markers of aneuploidies in twin pregnancies.

#### P08.59

#### Region wide association study of human chromosome 15q14-22.1 locus with type 2 diabetes in Japanese

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Several previous linkage scans in type 2 diabetes (T2D) families indicated a putative susceptibility locus on chromosome 15q14-22.1, while the underlying gene for T2D has not yet been identified. The presence of T2D disease susceptibility variant(s) was assessed in the 21.8 Mb region between *D15S118* and *D15S117* in a Japanese population using a region-wide case-control association test. A two-stage association test was performed using Japanese subjects: The discovery panel (Stage 1) used 372 cases and 360 controls, while an independent replication panel (Stage 2) used 532 cases and 530 controls. A total of 1,317 evenly-spaced, common SNP markers with minor allele frequencies  $> 0.10$  were typed for each stage. Captured genetic variation was examined in HapMap JPT SNPs, and a haplotype-based association test was performed. SNP2140 (rs2412747) (C/T) in intron 33 of the ubiquitin protein ligase E3 component n-recognin 1 (*UBR1*) gene was selected as a landmark SNP based on repeated significant associations in Stage 1 and Stage 2. However, the marginal  $p$  value ( $p = 0.0043$  in the allelic test, OR = 1.26, 95% CI = 1.07-1.48 for combined samples) was weak in a single locus or haplotype-based association test. We failed to find any significant SNPs after correcting for multiple testing. The two-stage association test did not reveal a strong association between T2D and any common variants on chromosome 15q14-22.1 in 1,794 Japanese subjects. A further association test with a larger sample size and denser SNP markers is required to confirm these observations.

#### P08.60

#### Genetic association between single nucleotide polymorphisms of *PAX5* and systemic lupus erythematosus in Hong Kong Chinese

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Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease characterized by heterogeneous manifestations and involvement of complex genetic and environmental components. Our genome-wide scan with 314 cases and 920 controls by Illumina 550K bead-chip has

revealed that single nucleotide polymorphisms (SNPs) of a novel gene, *PAX5*, confer association with SLE ( $P < 5 \times 10^{-3}$  in basic allelic test). Among all susceptibility SNPs, rs7859667, rs280025 and rs2812324 confer independent contribution upon logistic regression and conditional haplotype-base analysis ( $P < 0.05$ ), whose risk alleles constitute a risk haplotype locating upstream of *PAX5* ( $P = 2.05 \times 10^{-4}$ ). Further, rs7859667 is selected for replication in 754 SLE samples and 1032 controls by TaqMan, and the association is validated (OR = 1.16,  $P = 0.012$ ). The joint analysis of gene chip and replication data reveals SLE association with an OR of 1.18 and  $P$  value of  $5 \times 10^{-3}$ . Fine mapping of *PAX5* locus has been performed by selecting tag SNPs from 100 kb downstream to upstream of the gene and the genotyping results are pending. *PAX5* encodes a B-cell-specific activator protein that binds to promoters of the CD19 gene, *BLK*, and to regulatory regions of the immunoglobulin heavy chain locus, affecting B cell development and proliferation. Thus genetic and biological evidence are both suggesting a genuine association between *PAX5* and SLE.

#### P08.61

#### TNF $\alpha$ (-308G/A) promoter polymorphism in systemic sclerosis patients from Romania

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**Background:** It is shown that TNF $\alpha$  participates in activation of vascular endothelium, regulation of immune response and metabolism of the connective tissue by modulation of fibroblastic function. Systemic sclerosis (SSc) patients exhibit a systemic and local rise of TNF $\alpha$  content. This rise contributes to SSc progression development of fibrosing alveolitis and skin fibrous alterations in Raynaud's syndrome.

**Objectives:** The aim of this study was to investigate one of TNF $\alpha$  most studied promoter polymorphism (-308 G/A) in SSc patients from Romania.

**Methods:** 37 unrelated patients with SSc diagnosed by a qualified rheumatologist (35/2 F/M) and 67 healthy unrelated organ donors (41/26 F/M) were typed for TNF $\alpha$  -308G/A polymorphism (rs 1800629) by TaqMan SNP Genotyping Assay C\_7514879\_10 (Applied Biosystems, USA).

**Results:** The observed genotypes for the TNF $\alpha$ -308G/A polymorphism (2.70% AA, 13.51% GA, 83.78% GG in SSc patients, respectively 4.47%AA, 19.40%GA, 76.11%GG in controls) showed no departure from Hardy-Weinberg equilibrium (HWE).

**Conclusion:** The present study shows no departure from HWE in SSc patients from Romania and no potential association of investigated allele with the susceptibility to this disease. In order to improve the statistical power of this study, a larger number of patients may be required to verify this conclusion.

#### P08.62

#### Translocations and inversions in Finland

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Finland is acknowledged of its high standard of clinical medicine and in the disease gene hunt of its founder populations. What is perhaps not so well recognized is that Finland has probably the most comprehensive health registers and records: hospitalizations, surgeries, chronic diseases, and prescriptions etc. have been filed for decades.

Relying on this infrastructure we are gathering information on ~ 3000 known reciprocal balanced translocations and inversions to a national database ([www.fintransloc.org](http://www.fintransloc.org)). By analyses of the medical records and by searches of national registers, we are obtaining novel information of not only monogenic traits with unidentified mutations, but also of

multifactorial traits associated with any given chromosomal abnormality. Moreover, such a database will greatly assist genetic counseling efforts.

To date, we have surveyed 1529 hospital contacts involving translocations or inversions consisting of 395 families plus singletons. We are already breakpoint mapping three families providing potential shortcuts in the identification of disease genes.

Some interesting families				
Family	Chromosomal abnormality	Trait	Carriers	Traits of the carriers
5	t(1;12)	Specific delay in development	6	2 with specific delay, 1 with dyslexia, 2 with learning difficulty
29	t(2;18)	Dyspraxic developmental speech disorder	7	3 with speech difficulty, 1 with dyslexia
82	t(5;12)	Fibroma molle in palate	3	3 with fibroma molle (+ 1 patient with chromosome status unknown)
107	inv 8	Borderline mental retardation	16	11 with borderline mental retardation
128	t(10;11)	Aortic dilatation	2	1 aortic dilation (+ 2 patients with chromosome status unknown)
207	t(4;12)	Height	2	2 with growth disturbance (+ 1 individual with chromosome status unknown)

#### P08.63

#### Polymorphisms in KLF11 gene and development of type 2 diabetes in Japanese

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Kruppel-like factor 11 is a pancreatic transcription factor whose activity induces the insulin gene. In North European populations, its common functional variant Q62R (rs35927125) is a strong genetic factor for Type 2 diabetes ( $P=0.00033$ , odds ratio for G allele=1.29, 95% CI 1.12-1.49). We examined the contribution of *KLF11* variants to the susceptibility to Type 2 diabetes in a Japanese population. By resequencing Japanese individuals ( $n=24$ , partly 96), we screened all four exons, exon/intron boundaries and flanking regions of *KLF11*. Verified single nucleotide polymorphisms (SNPs) were genotyped in 731 initial samples (369 control and 362 case subjects). Subsequently, we tested for association in 1087 samples (524 control and 563 case subjects), which were collected in different districts of Japan from the initial samples. We identified eight variants, including a novel A/C variant on intron 3, but no mis-sense mutations. In an association study, we failed to find any significant result of SNPs (minor allele frequency 8.2-46.2%) after correcting for multiple testing. Similarly, no haplotypes were associated with Type 2 diabetes. It is notable that the G allele in rs35927125 was completely absent in 1818 Japanese individuals. Genetic variants in *KLF11* are unlikely to have a major effect of Type 2 diabetes in the Japanese population, although they were significantly associated in North European populations. These observations might help to determine the role of *KLF11* variants in Type 2 diabetes in different populations.

#### P08.64

#### Identification of genetic susceptibility markers of the TPH1 gene for unipolar depression

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The tryptophan hydroxylase isoform 1 (TPH1) gene is of interest with respect to the risk of unipolar depression (UD) as it expresses a biosynthetic enzyme for serotonin in the brain during development. Previous studies showed that the TPH1 gene to be associated with suicidal behavior, bipolar disorder. This study examined association of the polymorphisms A218C in intron 7 and A-6526G in the promoter region of TPH1 gene with UD in patients from Russian. Samples of 201 patients and 270 healthy volunteers were investigated using PCR method and subsequent enzyme digestion. We found significant differences in the genotype frequencies distribution ( $\chi^2=11.43, P=0.003$ ) of the A218C polymorphism between patients and control groups. An increase of the \*A/\*A genotype (OR=1.91, 95%CI 1.18-3.1) frequency and decrease of the \*A/\*C genotype (OR=0.55, 95%CI 0.38-0.81) frequency were registered in the depressive group compared to those in the control one. There were no statistical differences registered between UD patients and healthy controls in the genotypic and allelic distribution of the A-6526G polymorphism investigated. Maximum likelihood analysis of

haplotype distribution demonstrated the presence of linkage disequilibrium between the A218C and A-6526G polymorphisms both in control subjects ( $D'=0.85$ ) and in cases ( $D'=0.60$ ). Analysis of distribution of the haplotype frequencies revealed significant difference between depressive and healthy subjects ( $\chi^2=10.06, df=3, p=0.02$ ). Further analysis showed decrease of the haplotype AG ( $OR=0.35, 95\% CI 0.17-0.74$ ) in patient compared to control one. Our findings indicate the contribution of the TPH1 gene to susceptibility for UD. The research supported by the Russian Humanitarian Research Fund (#08-06-00579a) and Russian Science Support Foundation.

#### P08.65

#### Uniparental Disomy Analysis Using Linkage Mapping Set Markers

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In a departure from classical Mendelian genetics, there are a few imprinted genes that are only expressed from the allele inherited from one parent. Uniparental disomy (UPD) occurs when an individual inherits both copies of a chromosome pair from only one parent and no copies from the other parent. Individuals with UPD that have a deficiency in the expression of imprinted genes on Chromosome 15 are subject to two genetic disorders, Prader-Willi syndrome (PWS) and Angelman syndrome (AS). One method used to determine the presence or absence of the maternal or paternal chromosome is to interrogate the DNA from the individual and both parents by microsatellite or STR (Short Tandem Repeat) analysis with fluorescently labeled primers. We demonstrate new methods to compare the DNA via fragment analysis by capillary electrophoresis (CE). Such methods could help in producing consistent results across a wide range of laboratory environments. We also present a software analysis procedure to rapidly generate results and draw conclusions.

#### P08.66

#### Genetic analysis of Iranian families with Usher syndrome type 1, 2, 3

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Usher syndrome (USH) is an autosomal recessive disorder with sensorineural hearing loss, retinitis pigmentosa and in some cases vestibular dysfunction. At least 13 chromosomal loci are assigned to three clinical USH types, namely USH1A-H, USH2A-D and USH3A. The gene products of nine identified USH genes belong to different protein classes and families. There are five known USH1 molecules: myosin VIIa (USH1B); cadherin 23 (USH1D) and protocadherin 15 (USH1F); harmonin (USH1C) and SANS (USH1G). In addition, three USH2 genes and one USH3A gene have been identified. The three USH2 genes code for the transmembrane protein USH2A (usherin); the G-protein-coupled 7-transmembrane receptor, VLGR1b (USH2C); and (WHRN) USH2D. The USH3A gene encodes clarin-1. Worldwide USH1 and USH2 account for most Usher syndrome cases with rare occurrences of USH3. In the present study, we determined haplotype segregation in 33 consanguineous Iranian families using short tandem repeat polymorphic markers to examine all identified loci for USH1 (USH1A, USH1B, USH1C, USH1D, USH1E, USH1F, USH1G), all loci for USH2 (USH2A, USH2B, USH2C, USH2D) and USH3A. We found 10 families demonstrating haplotype segregation consistent with linkage to one of the loci related to USH1 or USH2: three families linked to USH1D, two families linked to USH1B and USH2C, and single families linked to USH1C, USH1F and USH2A. Identified mutations have included: MYO7A, R150X; VLGR1b, (g.371657\_507673del) and Arg155X. Mutation analysis of the other genes is being completed. This study suggests that mutations in *CDH23*, *MYO7A* and *VLGR1b* are major components to Usher syndrome in the Iranian population.

#### P08.67

#### Vitamin D receptor gene polymorphism and bone mineral density in children with intensive caries in St.Petersburg.

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**BACKGROUNDS:** Low bone mineral density (BMD) for chronological age, is well-known phenomena in children with intensive caries (IC). The bone abnormalities in these patients include rampant dental caries, extensive caries, and hypoplasia of the enamel, bone mineralization disturbances and growth retardation. Previous studies have suggested an effect of vitamin D receptor (VDR) alleles on bone mineralization (peak bone mass) and metabolism in patients with intensive caries.

**OBJECTIVE:** to investigate the relationship between VDR gene polymorphism and BMD in IC children.

**PATIENTS AND METHODS:** 82 IC children. Average age was  $11.7 \pm 2.3$  years. In all children we evaluated some markers of bone metabolism, osteocalcine, calcitonin, parathyroid hormone and markers of bone mineralization, Ca,  $Ca^{2+}$ , phosphate, and total alkaline phosphatase. TaqI and Apal polymorphism of the VDR gene was tested by PCR. BMD was identified by dual-energy X-ray absorptiometry of lumbar spine. Low BMD for chronological age was detected if Zscore  $< -2.0$  SD, in accordance to International Society for Clinical Densitometry.

**RESULTS:** we have identified patients with normal BMD - 42 and 40 patients with low BMD. We have revealed a significant difference in VDR genotype distribution between patients with normal and low BMD. TagI polymorphisms VDR were as follow TT-45.0% and 21.4%, Tt-47.5% and 61.9%, tt-7.5% and 16.7% ( $p < 0.01$ ). Apal polymorphism VDR were as follow AA - 5.0% and 26.2%, Aa-55.0% and 50.0%, aa-40.0% and 23.8%, respectively ( $p < 0.01$ ).

**CONCLUSION:** children with intensive caries with TT and aa genotypes of VDR have low BMD.

#### P09. Complex traits and polygenic disorders

##### P09.001

##### Clinical and molecular characterization of patients with acute toxic hepatitis

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Detoxifying enzymes activity changes can induce acute toxic hepatitis (ATH). One of the most frequent ATH causes is alcohol.

Aims. Identification of the most relevant gene polymorphisms (gene *UGT1A1* allele *UGTA1A\*28*, *GSTT1* and *GSTM1* null genotypes, *GSTP1* - A313G, *GSTA1* - C69T) in ATH patients and control group. Material: 51 ATH patients and 224 individuals in control group.

Methods: Genomic DNA was extracted and polymorphisms were analyzed using multiplexPCR, PCR-RFLP, sequencing.

Results. Frequencies of polymorphisms in ATH; control group ( $p$  value) are following: *UGT1A1\*28* - 0.38; 0.41 ( $p=0.43$ ), 69T 0.46; 0.34 ( $p=0.042$ ), 313G - 0.32; 0.37 ( $p=0.79$ ), *GSTT1* null genotype 0.14; 0.10 ( $p=0.47$ ), *GSTM1* null genotype 0.65; 0.56, ( $p=0.270$ ).

Clinical outcome-13 patients (25%) died of liver failure. Difference between patient groups, one - patients who died, in other who survived, molecular data was following - *UGTA1A\*28* - 0.30; 0.40 ( $p=0.62$ ), *GSTA1* 69T 0.38; 0.49 ( $p=0.62$ ), *GSTP1* 313G 0.27; 0.36 ( $p=0.71$ ), *GSTT1* and *GSTM1* double null genotype 0.15; 0.0 ( $p=0.014$ ). In patients medium level of total bilirubin was  $434 \pm 203$  mol/l - patients who has no *UGT1A1\*28* allele medium bilirubin level was  $360 \pm 187$  mol/l (with *UGT1A1\*28* 476  $\pm 202$  mol/l ( $p=0.057$ )). Total bilirubin level in patients with 313G allele was higher ( $471 \pm 174$  mmol/l), comparing with those who were 313A homozygous ( $p=0.08$ ).

Conclusions. *GSTA1* 69T allele is more frequent in ATH patients than in control group. *UGTA1A\*28* and 313G allele has impact to bilirubin level in ATH patients. *GSTT1* and *GSTM1* null genotypes could promote lethal outcome in acute toxic hepatitis.

## P09.002

## The role of Y chromosome in male susceptibility to ADHD and schizophrenia

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Many psychiatric disorders display distinct sex differences. Men are more likely to be affected by neurodevelopmental disorders, such as attention deficit hyperactivity disorder (ADHD) and schizophrenia. ADHD is a childhood disorder characterised by inattention, overactivity and impulsiveness. The disorder affects males more than females at a ratio of 4:1. In schizophrenia, the age of onset in men is significantly earlier (21 years) than for women (25 years). The disorder is more severe in male patients and they generally have more negative symptoms.

To elucidate the genetic causes of sex differences we choose to study the Y chromosome. Y chromosome variants appropriate for U.K. populations were genotyped in a sample of 210 cases with ADHD, 310 cases with schizophrenia and 700 U.K. controls. Although our case-control study did not reveal any association of Y chromosome haplogroups with either ADHD or schizophrenia, a modifying effect of Y chromosome on ADHD was shown. Scores of performance IQ and full scale IQ were found to be increasing as Y chromosome haplogroups became more recent evolutionary.

Performance IQ (p=0.014)		
Haplogroups (from most ancient to most recent evolutionary)	N	Mean
1	59	87.3
2	108	91.1
3	35	95
Full Scale IQ (p=0.024)		
Haplogroups (from most ancient to most recent evolutionary)	N	Mean
1	59	86.2
2	108	89.3
3	35	92.6

To sum up, Y chromosome haplogroups might not have a main effect on ADHD or schizophrenia but they have a modifying effect on ADHD since more ancient haplogroups are associated with lower performance IQ. Moreover, our results on 1220 individuals provide an insight into the population structure of U.K. Y chromosome haplogroups.

## P09.003

## Genetic variation within adiponutrin is associated with lipoprotein metabolism and liver function

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Objective. Adiponutrin (*PNPLA3*) is a predominantly liver-expressed transmembrane protein with phospholipase activity regulated by fasting and feeding. Recent genome-wide association studies identified adiponutrin to be associated with hepatic fat content and liver function, indicating that adiponutrin might be involved in hepatic lipoprotein metabolism. We aimed 1) to elucidate the association of common variants within adiponutrin and parameters of lipoprotein metabolism and 2) with liver function in up to four independent West-Eurasian study populations including up to more than 6000 individuals.

Methods. This study is based on the population-based Bruneck Study (n=800), the population-based KORA F3 Study (n=1644), the SAPHIR Study (n=1738) from Austria based on a healthy working population, and the Utah Obesity Case-Control Study (including 1037 severely obese individuals (average BMI 46.0 kg/m<sup>2</sup>) and 827 controls from the same geographical region).

Results. We observed a strong recessive association of a common nonsynonymous variant within adiponutrin (rs738409, exon 3) with age- and gender-adjusted lipoprotein concentrations which was not caused by an impaired liver function: homozygote carriers of the mi-

nor allele had on average 8.75 mg/dL lower total cholesterol levels (p=0.00006), 7.79 mg/dL lower non-HDL cholesterol levels (p=0.0004), 4.91 mg/dL lower LDL cholesterol levels (p=0.007) and 9.01 mg/dL lower triglyceride levels (p=0.006). Moreover, the rs738409 variant is strongly associated with the liver enzymes ALT and AST, following a recessive model. Discussion. In conclusion, our study suggests that adiponutrin is related to processes of lipid and energy metabolism with a special focus on apolipoprotein B-containing lipoproteins, as well as hepatic dysfunction.

## P09.004

Dystrobrevin binding protein 1 gene (*DTNBP1*) and bipolar disorder: the results of a meta-analysis

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Recent studies suggest an overlap in genetic susceptibility of schizophrenia and bipolar disorder (BD). There is some evidence for association between the *DTNBP1* gene with schizophrenia and to date there have been five association studies of *DTNBP1* and BD published: Raybould et al (2005), Breen et al (2006), Joo et al (2007), Pae et al (2007), and Gaysina et al (2008). The main limitations of these studies are an impossible direct comparison of risk alleles/haplotypes due to different marker sets used and their insufficient sample size. To deal with these problems we imputed the genotypes for Hapmap SNPs for samples used in studies by Breen et al. (2006) and Gaysina et al. (2008). Accuracy over 90% of imputed data makes them appropriate for analyses of genotypes and alleles, but not haplotypes. If the data were available from three or more studies we performed a meta-analysis of *DTNBP1* alleles combining odds ratios and using a fixed-effect model. Seven SNPs were available for the meta-analyses: rs2619538, rs2619522, rs760761, rs2005976, rs1011313, rs3213207 and rs2619539. Our results support the association of rs760761 (T allele: P (Z)=0.012, OR=1.22, 95%CI 1.04-1.42) and rs3213207 (G allele: P (Z)=0.013, OR=1.24, 95%CI 1.05-1.46) of the *DTNBP1* gene in vulnerability to BD. We are currently running meta-analyses based on Bayesian method which allows combining data of genetic association studies with different sets of markers (Verzilli et al, 2008). The data will be presented during the conference.

## P09.005

## DNA-variants of the properdin gene are not associated with AMD

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Purpose. Several variants in the complement cascade genes (complement factor H [CFH], C2, C3, CFB) have been reported to associate with age-related macular degeneration (AMD). Of them, a member of the complement alternative pathway, CFH, represents the highest risk. Since properdin is also an important protein in this pathway, we analysed whether DNA-variants in the properdin gene at Xp11.4 are associated with AMD.

Methods. The coding region and splice sites of the properdin gene were sequenced in a total of 223 Finnish patients with AMD (151 sporadic cases [68% women] and 72 familial cases [71% women]). Controls were 86 age-matched non-AMD patients (81% women) with no large drusen and no or minimal focal pigmentary abnormalities.

Results. A total of four single nucleotide polymorphisms (SNP) were detected in the properdin gene. Three of the SNPs (D55H in exon 3, D299N in exon 7 and P394S in exon 9) were infrequent (in 5 patients and controls in total). The fourth SNP, rs1048118 in exon 10, was more frequent, but was not associated with AMD either in men or in women in our preliminary analyses.

Conclusions. None of the variant in the properdin gene was associated with AMD, suggesting that properdin, though a member of alternative pathway, does not play as important role as CFH in the pathogenesis of AMD.

## P09.006

## Association of ADH genes variation with alcoholism risk in three populations from Russia

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Alcoholism (alcohol dependence) is a common, complex disease, with significant genetic contribution to the risk. Alcohol is primarily degraded by alcohol dehydrogenase (ADH) and genetic variations affecting the rate of alcohol degradation were found in ADH genes.

We designed a classical case-control association study for 5 polymorphisms in Class 1 ADH genes: *ADH1B Arg47His* (rs1229984), *ADH1B RsaI* (rs2066701), *ADH1C HaeIII* (rs1693425), *ADH1C EcoRI* (rs1789920), and *ADH1C Ile349Val* (rs698). We recruited 303 men with ICD-10 alcoholism diagnosis (112 Russians, 91 Tatars, 100 Bashkirs from Volga-Ural region of Russia) and matched control groups typed for the above-mentioned gene variants using PCR-RFLP technique. We observed all SNPs to be in strong linkage disequilibrium in controls, cases and the overall sample in all studied populations.

Haplotype analysis of 2 SNPs in *ADH1B* gene showed significant association of *ADH1B\*Arg\*T* haplotype with alcoholism ( $p<0.05$ , OR=1.60) in Russians. Analysis of 3 polymorphisms in *ADH1C* gene revealed that in Tatars the frequency of individuals carrying *ADH1C\*T\*G\*G* haplotype was significantly higher in the group of alcoholics compared to healthy controls ( $p<0.05$ , OR=4.93). In Bashkirs the frequency of haplotype *ADH1C\*G\*G\*A* was significantly increased in patients with alcoholism compared to control individuals ( $p<0.05$ ). Further analysis demonstrated association of this haplotype with the risk of alcoholism in Bashkirs (OR=12.20).

Our findings indicate the contribution of *ADH1B* and *ADH1C* genes to susceptibility for alcoholism in three populations from Russia. Further investigations of these genes in different phenotypic groups are necessary. This work was supported by Russian foundation for humanities grant (08-06-00579a).

## P09.007

## Functional variants of the serotonin receptor type 3A and B gene are associated with eating disorders

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As a key player in modulating both human physiological and behavioural functions including anxiety, perception and in particular appetite, serotonin is likely to be involved in the aetiology of eating disorders. Bulimia nervosa patients have been effectively treated with the selective 5-HT3 antagonist ondansetron. This triggered our interest in investigating the putative role of variants in the 5-HT3 receptor genes HTR3A and HTR3B in the susceptibility to bulimia nervosa (BN) and anorexia nervosa (AN) by direct sequencing. 265 patients with AN and 91 patients with BN as well as 191 healthy controls served as a pilot study group for mutational analysis. We found the coding HTR3B variant p.Y129S (rs1176744,  $P = 0.004$ , OR = 2.06) and the 5'UTR residing HTR3A variant c.-42C>T (rs1062613,  $P = 0.008$ , OR = 5.31) to be associated with the restrictive subtype of anorexia nervosa (ANR). An intronic HTR3A variant, IVS1-19G>A (rs1176722), was identified to be associated with the ANR ( $P = 0.002$ ) as well as the BN purging subtype (BNP,  $P = 0.005$ ). Furthermore, the association of HTR3B p.Y129S with ANR was confirmed in an independent Spanish study group of 78 patients with AN and 331 controls ( $P = 0.034$ , OR = 2.26). Hence, our study provides first evidence for an involvement of serotonin receptor type 3 variants in the aetiopathology of eating disorders in humans.

## P09.008

## Study of association between ApoE genotype and cognitive performance in young adults

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It is well known that presence of ApoE ε4 allele is strongly associated with early onset and rapid course of Alzheimer dementia. Although the exact role of ApoE in dementia is not clear, recent data suggest both direct and indirect mechanisms. In addition, association between ApoE genotype and cognitive performance in whole population varies at different ages, and impact of ApoE ε4 allele on brain function in young persons is still controversial.

The aim of our study was to determine ApoE genotypes in a group of healthy young adults and to investigate association of determined genotypes with their cognitive performances.

Our study was carried out on 548 students of School of Medicine, Belgrade. ApoE (ε2/ε3/ε4) polymorphisms were determined by polymerase chain reaction (PCR) and RFLP analysis on polyacrylamide gel. Cognitive screening was performed by means of Mini-Mental State Examination (MMSE).

We detected ε2/ε3 genotype in 55 (10,0%), ε2/ε4 in 6 (1,1%), ε3/ε3 in 426 (77,74%), ε3/ε4 in 57 (10,4%) and ε4/ε4 in 4 (0,73%) students. Statistical analysis showed significantly lower MMSE score in persons caring ε4 allele either in homozygous or in heterozygous state. Our results suggest that ApoE genotype has significant role in cognitive performance even at young age.

## P09.009

## Towards a finer evaluation of the role of IL2RA in Multiple Sclerosis

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IL2RA on chromosome 10 is a well-established risk factor for Multiple Sclerosis (MS). First found in a genome-wide study of UK and US families (IMSGC, 2007), it has been replicated in several European populations (Weber, Fontaine et al, 2007; Matesanz et al 2007; IMSGC, 2008). However, all these studies focused only on single SNP association. So, the odds-ratios reported in the literature do not correctly reflect the differential genotypic risks attributable to IL2RA as a whole. In order to better discriminate the relative risks attributable to IL2RA genotypes, we undertook finer mapping of this gene in the REGENSEP collection of 563 trio families with 28 tagSNPs. A 10-SNP subset, at the 5' end of the gene was retained as showing the best contrast between cases and controls. All combinations of SNPs within this subset were tested for association. This procedure led to retain a combination of 2 SNPs, rs3118470 and rs2256774. Note that, taken individually, rs2256774 was not shown to be associated. However, it strongly reinforces the association signal observed with rs3118470. This 2-SNP combination corresponds to 10 distinct phased genotype possibilities, the relative risks of which range from 1 to 3.34.

Simultaneous analysis of variants in a given gene provides an important information on its role in the etiology of a complex disease such as MS. However, it is only a preliminary step towards bridging the gap between the association signal to the evaluation of the gene effect, and towards understanding the full biological pathway.

## P09.010

## Catecholamine O-Methyltransferase (COMT) gene polymorphisms are not associated with multisomatoform disorder (MSD) in a group of German MSD patients and healthy controls

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Background: Multisomatoform Disorder (MSD) is characterized by the presence of 3 or more medically unexplained, currently bothersome, physical symptoms with an at least 2 year history of somatization. The

ethiopathology of MSD is largely unknown, but genetic disposition may be one of several risk factors. Since pain is a major symptom of MSD, and polymorphisms in the catecholamine O-Methyltransferase (COMT) gene are associated with COMT enzymatic activity and pain sensitivity, we assumed that COMT polymorphisms could be associated with MSD.

Probands: 147 patients with MSD and 94 age and gender matched healthy controls participated in this study. The inclusion criteria for MSD were in accordance to the structured clinical interview (SCID) of the diagnostic and statistical manual of mental disorders (DSM IV).

Genotyping: DNA from EDTA blood was genotyped for single nucleotide polymorphisms (SNPs) within the COMT locus by polymerase chain reaction (PCR) and restriction enzyme analysis. The distribution of COMT SNP alleles, genotypes and haplotypes was compared between patients and controls.

Results: None of the investigated SNPs, including the functionally relevant common SNP in codon 158 (Val158Met), showed a statistically significant allelic, genotypic or haplotypic association with MSD.

Discussion: In previous studies, convincing evidence was obtained for a contribution of single SNPs and SNP haplotypes of the COMT locus to differences in the human experience of pain and to the risk of developing temporomandibular disorder (TMD). In contrast to these studies, COMT polymorphisms do not seem to play a relevant role as major genetic risk factors for MSD.

#### P09.011

#### Association analysis of xenobiotic-metabolizing gene polymorphisms with asthma in Volga-Ural region of Russia

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Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The pathogenesis and etiology of asthma are very complex. Some studies have shown an association between asthma and polymorphisms of enzymes that play an important role in the biotransformation of xenobiotics. Using allele-specific hybridization on the biochip we have investigated the allele and genotype distribution of 13 polymorphisms in eight genes (CYP1A1, CYP2D6, GSTT1, GSTM1, MTHFR, NAT2, CYP2C9 and CYP2C19). The study was performed in 264 patients with bronchial asthma and 201 nonasthmatic individuals from Volga-Ural region of Russia. Biochips were prepared in Engelhardt Institute of Molecular Biology, Russian Academy of Sciences (Biochip-IMB, Russia). Significant differences in NAT2 allele and genotype frequencies have been found between asthma patients and control group of Russian ethnicity. The frequency of slow \*5/\*5 genotype was lower in asthma patients than in healthy subjects (OR=0.49; 95%CI 0.26-0.92; p=0.026). On the contrary the Tatars had significant higher frequencies of \*5 allele and \*5/\*5 genotype of NAT2 gene in patients than in control group (OR=1.54; 95%CI 1.01-2.35; p=0.045; OR=3.29; 95%CI 1.31-8.23; p=0.008). It is known that polymorphic NAT2 status varies widely between individuals and ethnic groups. Both rapid and slow N-acetyltransferase genotypes have been associated with the risk of several diseases in a number of populations. These observations, together with ethnic variation in the ratio of slow and rapid acetylators, suggest that NAT2 genotypes may partially explain asthma risk in different populations in Volga-Ural region of Russia.

#### P09.012

#### ORMDL3 haplotype is associated with asthma in an Italian familial collection.

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Asthma is a complex disease with genetic and environmental components.

In 2007 the first Genome Wide Association study for asthma in English and German subjects (Moffat MF, Nature 2007;448:470) reported a

strong association with 17q21 locus including the ORMDL3 gene. This finding has been already replicated in different populations.

The association of 4 SNPs (rs8067378, rs7216389, rs3859192, rs11650680) in the candidate locus with susceptibility to asthma was investigated in Italian families with young asthmatic individuals (<16 years old).

A family-based data set composed of 211 Italian families ascertained through an allergic asthmatic child was analyzed (previously described: Malerba J Allergy Clin Immunol 2001; 107: 654).

Single SNP and haplotype analyses were computed using the computer program UNPHASE. Single SNP analyses and haplotype analyses considering the 4 SNPs did not show significant result, but a significant association for haplotype rs7216389-rs3859192 T-C and asthma was found (global test p=0.007; OR=1.59 p=0.008).

In conclusion, we confirm the association of 17q21 region with young asthmatic individuals in a different population, and we extend it to ORMDL3 gene marker haplotypes.

#### P09.013

#### Polymorphisms of ACE and TFAM genes and left ventricular remodeling in athletes

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Left ventricular (LV) hypertrophy in endurance-oriented athletes is generally understood to be a limiting factor for improving maximal oxygen uptake. LV growth is regulated by several independent signaling pathways. These include pathways involved with the RAAS (rennin-angiotensin-aldosterone system), PGC1/TFAM (mitochondrial biogenesis, fatty acid oxidation), etc. Several studies demonstrated an association between LV hypertrophy and the presence of ACE DD genotype. Mitochondrial transcription factor A (TFAM) is essential for mtDNA transcription and replication. Myocardial mtDNA copy number and TFAM expression both decreased in several cardiac diseases. However, the functional significance of TFAM has not been established in LV growth. The purpose of the study was to investigate ACE I/D and TFAM Thr12Ser polymorphisms for association with echocardiographic measures in female athletes. Fifteen elite female athletes (all-round speed skaters and rowers) were studied. ACE and TFAM gene polymorphisms were determined by PCR-RLFP. Echocardiography was performed for the measurement of left ventricular mass, wall thickness, dimensions and function. We found that LV mass was significantly greater in TFAM SS homozygotes than in T allele carriers (SS - 200 (26) g, ST - 149 (30) g; P=0.034). Furthermore, the interventricular septal wall thickness was biggest in ACE DD homozygotes than in I allele carriers (DD - 0.88 (0.15) cm, ID - 0.83 (0.16) cm, II - 0.7 (0) cm; P=0.047). In conclusion, ACE II (with lower ACE activity) and TFAM ST (with higher transcription activity) genotypes are associated with lower risk for development of left ventricular hypertrophy in female athletes.

#### P09.014

#### Genetic predisposition of young athletes to metabolic disorders

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The purpose of the present work was to estimate the risk for development of metabolic disorders (type 2 diabetes, coronary heart disease, obesity) by detecting polymorphisms of genes in young Russian athletes. Two hundred and thirty two sub-elite and elite athletes (118 bicyclists, 75 wrestlers and 39 rowers; 13-20 yr) were participated in the study. Polymorphisms of PPARA (intron 7 G/C), PPARG (Pro-12Ala), UCP2 (Ala55Val), UCP3 (-55C/T); genes were determined by PCR-RLFP. The concentration of triglycerides, total cholesterol and cholesterol of high density lipoproteins, glucose, glycated hemoglobin and homocysteine were determined in serum after 12-hour overnight fasting. We found that 33.2% of young athletes had a high risk for development of cardiovascular disorders, type II diabetes, obesity and atherosclerosis. None of the studied biochemical parameters and anthropometric measures was associated with genotypes, indicating that high physical activity regardless of the carriage of unfavorable genotypes may improve metabolic profile of athletes. Evaluation of

young athletes' nutrition status showed that the level of dietary fat was 30% of total energy intake due to fat-rich diet and low carbohydrate consumption. There was a deficiency in A, B1, B2, B6 vitamins and calcium intake, whilst cholesterol consumption was high in athletes. In conclusion, combining information from several known common polymorphisms allows the identification of athlete subgroups (33.2% in our study) with markedly differing risks of development of metabolic disorders. High physical activity, rational nutrition of athletes and well-balanced diet are important factors which may modulate negative effects of unfavorable genotypes.

#### P09.015

#### NFATC4 Ala160Gly polymorphism and elite power athlete status

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The nuclear factor of activated T-cell (NFAT) family of transcription factors contributes to the development of several organ systems. Mutation of NFAT genes affects the development of endothelial cells, skeletal myoblasts, chondroblasts, keratinocytes, and adipocytes. NFATC4 effects changes in gene expression during hypertrophy and fiber-type switching in cardiac and skeletal muscle. We have recently shown that Gly160 allele of the functional Gly160Ala polymorphism in NFATC4 is associated with increased proportion of fast-twitch fibers of *m. vastus lateralis* in athletes. As muscles of elite sprinters and weightlifters predominantly consist of fast-twitch fibers, we therefore speculated that NFATC4 Gly/Gly homozygotes should be more prevalent within a group of power-oriented athletes. We have tested this hypothesis in a case-control study of 91 Russian power-oriented athletes and 1113 controls. Cases consisted of elite athletes involved in jumping events, speed skating (500-1000 m), swimming (50-100 m), throwing events and weightlifting. NFATC4 gene Gly160Ala polymorphism was determined by PCR-RLFP. We found that the frequencies of Gly/Gly genotype (36.3% vs. 19.9%;  $P=0.0005$ ) and Gly allele (59.3% vs. 44.1%;  $P<0.0001$ ) were significantly higher in athletes than in controls, indicating that Gly allele has favorable effect on power performance. In conclusion, NFATC4 Gly160Ala polymorphism is associated with elite power athlete status.

#### P09.016

#### Analyses of the individual and aggregate genetic contributions of previously identified polymorphisms of SPINK5, KLK7 and FLG to eczema risk

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Polymorphisms in the serine protease inhibitor gene serine peptidase inhibitor Kazal type 5 (SPINK5) and the serine protease kallikrein-related peptidase 7 (KLK7) appear to confer risk to eczema in some cohorts, but these findings have not been widely replicated. These genes encode proteins thought to be involved in the regulation of posttranslational processing of

filaggrin (FLG), the strongest identified genetic risk factor for eczema to date.

We sought to clarify the individual risk of eczema conferred by the SPINK5 polymorphism rs2303067 and a previously described insertion in the 39 untranslated region of KLK7 and to examine potential epistatic effects between these variants and FLG mutations.

We examined the effects of these polymorphisms and FLG in 486 German families,

287 German and 418 Irish/English eczema cases (n for 3 genes: 1191 vs 4544 controls). We then additionally studied the SPINK5 polymorphism and FLG mutations in 1583 eczema patients from the ALSPAC study (n for 2 genes: 2774 vs 10,607 controls).

No association was seen with the SPINK5 or KLK7 variants in the case-control analysis; however, a weaker effect was observed for the SPINK5 variant with maternal transmission in the family study. No interactions were seen between the polymorphisms in KLK7, SPINK5,

and FLG.

The SPINK5 420LysSer mutation confers a risk of eczema when maternally inherited but is not a major eczema risk factor. The KLK7 insertion appears to confer no risk of eczema. We found no interaction between the SPINK5 risk allele or the putative KLK7 risk allele and FLG mutations.

#### P09.017

#### Monogeneous inheritance in ADHD? Report from a fine-mapping study

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Attention-deficit hyperactivity disorder (ADHD) is a neuropsychiatric disorder characterized by symptoms of inattention, hyperactivity and increased impulsiveness. Despite many studies over the last decades, the etiology of ADHD remains unknown. Eight large ADHD-affected families were recruited for this study. These families were recruited in a multicenter collaboration study conducted by three clinical units of child and adult psychiatry (University of Trier, Homburg/Saar, and Wuerzburg). They comprised of 191 individuals, of which 95 were affected with ADHD.

Fine-mapping of the significant loci reported in a recent paper (Romanos *et al.* 2008) of a genome-wide linkage study of the eight large families was carried out. Using genetic data derived from related individuals within each of the large families, an attempt is made to identify predisposing chromosomal regions in these families.

From the preliminary results, in one of the large families, a 20 cM interval on chromosome 18 was narrowed down to 18q11- 18q21. All affected individuals in this family share the same haplotype.

ADHD is believed to be a complex, polygenic disease in which many genes of small effect contribute to disease susceptibility. However, there may be monogeneous Mendelian inheritance in large families. Currently, interesting candidate genes in the narrowed down region of chromosome 18 are being selected for further functional studies.

#### P09.018

#### A 2.2Mb microduplication in 1q42.2 including *DISC1* in 2 brothers with autism and mild mental retardation

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A growing number of copy number variations (CNV) are detected in individuals with neurodevelopmental disorders. However, the interpretation is not always straightforward.

We describe the identification and delineation of a 2.2Mb microduplication in 1q42.2 in 2 brothers with autism and mild mental retardation. The duplication was detected by Array-CGH with clones from the 1 Mb BAC/PAC clone set (Sanger Institute Hinxton, UK). The aberration was further delineated to 2.2Mb with a full-tiling BAC array. By means of quantitative real-time PCR (qPCR) the breakpoints of the duplication were mapped and segregation in the family was investigated. qPCR was used to screen 260 patients with autism for *DISC1* duplications.

The 2.2Mb duplication was present in the proband, his affected brother and the apparently unaffected father and paternal grandmother. Since this duplication was not present in 1577 Belgian persons, it was considered as a rare variant. Within this region the most interesting gene with respect to autism is *DISC1* (*disrupted-in-schizophrenia 1*) since it is known to be involved in schizophrenia and has recently been associated to autism and bipolar disorder. A group of 260 patients with autism was studied for the occurrence of *DISC1* duplications, but no additional duplications were found.

This study is a typical illustration of the difficult interpretation of causality of a rare variant in neuropsychiatric disease. We conclude that the *DISC1* duplication is a rare variant that probably confers susceptibility for autism in the current family.

**P09.019****Role of serotonin transporter promoter length polymorphism in autism: A South African population based study****Z. Arieff, M. Kaur, H. Gameeldien, M. Davids;***University of the Western Cape, Bellville, South Africa.*

The serotonin transporter promoter length polymorphism (5-hydroxytryptamine transporter length polymorphism, 5-HTTLPR) has long been implicated in autism and other psychiatric disorders. The use of selective serotonin reuptake inhibitors (SSRIs) have shown to have a positive effect in treating some symptoms of autism. The effects of these drugs vary in individuals due to the presence of S or L alleles of 5-HTTLPR. Studies performed on various autistic populations have found different allele frequencies for the L and S alleles. In the present study, allelic frequencies and genotypes of 110 South African (SA) autistic individuals (21 African, 48 Mixed and 41 Caucasian) were determined and compared with the matching SA ethnic control populations. The S/S genotype was found to be highly significantly associated with all the SA autistic ethnic populations namely, Caucasian,  $\chi^2 = 11.078$ ; Mixed,  $\chi^2 = 18.512$  and total group,  $\chi^2 = 46.712$  (df = 2;  $p < 0.001$ ). A highly significant increase in the S allele of the Mixed autistic group ( $\chi^2 = 14.877$ ,  $p < 0.001$ , df = 1) and the total autistic group ( $\chi^2 = 17.742$ ,  $p < 0.001$ , df = 1) was found when compared to the matching control groups. The comparison of our data with studies of other autistic populations round the world showed highly significant differences in allele numbers to French, Germans, Israelis, Portuguese and the Americans ( $p < 0.005$ ). It was less significant for the second French group, Japanese and Korean populations ( $p < 0.05$ ) and no difference was observed between the Indian autistic population. This is the first SA study of autistic individuals of different ethnic backgrounds showing significant differences of the allele and genotype frequencies of the 5-HTTLPR.

**P09.020****Identification of novel X-linked functional variants associated with autoimmune thyroid diseases****S. I. Gulsuner<sup>1</sup>, S. Gullu<sup>2</sup>, T. Ozcelik<sup>1</sup>;**

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Autoimmune thyroid diseases (AITDs) are more prevalent in females. We observed that a significant proportion of females diagnosed with AITDs display extremely skewed X chromosome inactivation (XCI) ratios in their blood cells (Eur. J. Hum. Genet. 14; 791-97, 2006). "Loss of mosaicism" for X-linked gene expression could be the first step of the cellular events that lead to the breakdown of self-tolerance in females. We propose that co-inheritance of two distinct events on the X: mutations that cause skewed XCI, and heterozygosity for non synonymous polymorphisms in as yet unknown but critically important genes could contribute to autoimmune processes. This hypothesis was tested with a custom made Affymetrix 5K microarray that we designed, which contained all known coding SNPs on the X, as well as intronic SNPs with high heterozygosity ratios. In addition, 166 autoimmunity associated autosomal SNPs (Nature 447; 661-78, 2007; Nat. Genet. 39; 857-64, 2007) were printed on the same microarrays. 84 female patients and 248 female controls with known XCI profiles were genotyped. The strongest associations were at nonsynonymous SNPs in TFPD3 [OR: 3.44(95% CI:1.62-7.29;  $p=0.00067$ )], ZMAT [OR: 2.22(95% CI: 1.23-3.66;  $p=0.003$ )], and C1GALT1C1 [OR:2.59(95% CI:1.16-2.59;  $p=0.006$ )]. Autosomal SNPs with significant associations were in SH2B3 [OR:2.03(95% CI: 1.42-2.91;  $p=0.00088$ )] and C12orf30 [OR: 1.99(95% CI: 1.39-2.85;  $p=0.00016$ )]. We note that X-linked SNPs reported here were not represented in the commercially available genome-wide association study platforms. These results suggest that the X chromosome could be critically important in female predisposition to AITDs. This work is supported by TUBITAK-SBAG-3334 grant.

**P09.021****Copy Number Variation in Bipolar Affective Disorder****D. Grozeva<sup>1</sup>, G. Kirov<sup>1</sup>, N. Norton<sup>1</sup>, D. Ivanov<sup>2</sup>, M. Owen<sup>1</sup>, M. O'Donovan<sup>1</sup>, N. Craddock<sup>1</sup>;**

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Copy number variation (CNV) in the human genome is very common and may play an important role in disease susceptibility. The role of CNVs in bipolar disorder (BD) has been largely unknown, in contrast to other common neuropsychiatric disorders, such as autism and schizophrenia, where a clear role for CNVs has been established in multiple publications.

The objective of the current study is to determine whether large (>100kb) and rare (found in <1% of the population) CNVs play a role in the susceptibility to BD.

We performed a genome-wide survey for CNVs in 1697 BD patients and 2806 healthy controls from the Wellcome Trust Case Control Consortium, using the Affymetrix 500K array.

The total burden of CNVs was not increased in patients when compared with controls. These results are in contrast with those obtained for schizophrenia patients analysed with the same methods in other studies, including those in our department, where we found an excess of CNVs >1Mb in size, when compared to both to the bipolar patients, or the same group of controls.

These results suggest that large rare CNVs may not play a substantial role in developing BD, unlike their confirmed role in schizophrenia and autism. This probably reflects the complex nature of the underlying genetic components leading to susceptibility to BD, involving many genes and genetic variants. CNV platforms with higher resolution and quality should be used to investigate the role of smaller CNVs in BD.

**P09.022****Genes for elite bodybuilder status****I. I. Ahmetov, A. M. Hakimullina, S. E. Khalchitskiy, R. R. Dondukovskaya, V. A. Rogozkin;***St Petersburg Research Institute of Physical Culture, St Petersburg, Russian Federation.*

Genetic influence on power performance and extremity circumferences is suggested by family and twin studies. The aim of the present study was to reveal an association between AMPD1 (regulates AMP metabolism) C34T, PPP3R1 (involved in hypertrophic response) 5I/5D, PPARG (regulates lipid metabolism) Pro12Ala, VEGFA (activates angiogenesis) G-634C gene polymorphisms and several physiologic and anthropometric parameters in bodybuilders. The study involved 21 highly elite male bodybuilders (World and Europe Championship winners and participants). Subjects were questioned about their best results in powerlifting squat, bench press and deadlift. For muscularity estimation four extremity circumferences were measured: extended upper arm (EAC), forearm (FC), calf (CC), and thigh (TC). Genotyping for the gene variants was performed by polymerase chain reaction and restriction enzyme digestion. We found that VEGFA C allele (with higher transcriptional activity) carriers exhibited the greatest relative muscle mass (CC/GC - 57.9 (3) %, GG - 53.9 (4.2) %;  $P=0.03$ ). PPP3R1 5D allele (known as hypertrophy-related allele) was associated with biggest values of EAC (5I/5D - 44 (5.5) cm, 5I/5I - 38.9 (3.7) cm;  $P=0.029$ ). AMPD1 CC homozygotes demonstrated greater results in deadlift than carriers of mutant (null) T allele (CC - 245 (42) kg, CT - 180 (0) kg;  $P=0.049$ ). Thus, the presence of AMPD1 C, VEGFA C and PPP3R1 5D alleles are associated with greater power performance and muscle mass gain in elite bodybuilders.

**P09.023****Analysis of variation in the canine melanocortin-4 receptor gene (mc4r)****L. van den Berg<sup>1,2</sup>, S. M. van den Berg<sup>3,4</sup>, E. E. C. P. Martens<sup>3</sup>, H. A. W. Hazewinkel<sup>3</sup>, N. A. Dijkshoorn<sup>5</sup>, H. A. Delemarre-van de Waal<sup>1</sup>, P. Heutink<sup>6</sup>, P. A. J. Leegwater<sup>3</sup>, H. C. M. Heuven<sup>3</sup>;**

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The melanocortin-4 receptor plays a central role in the regulation of energy balance in humans and other species. Rare mutations in the coding region of the gene encoding this receptor (MC4R) are the lead-

ing cause of monogenic obesity in humans. Common variants at this locus are associated with body mass index in the general human population. We hypothesized that common variants in the canine *mc4r* are associated with canine body mass. A cohort of 195 Golden Retriever dogs was used to investigate this hypothesis. The familial relationships, weight, length and height of these dogs were available. From these variables, we calculated a body index score as weight / (length \* height). The estimated heritability was 0.35 (s.e. 0.25) for weight, 0.15 (s.e. 0.21) for length, 0.32 (s.e. 0.26) for height, and 0.16 (s.e. 0.26) for body index score. We sequenced *mc4r* in 23 unrelated dogs from the cohort and detected four common polymorphisms: c.637G>T, c.777T>C, c.868C>T, and c.\*33C>G. Two of these were predicted to be deleterious by an *in silico* analysis using Polyphen. These polymorphisms were subsequently genotyped in the complete cohort. Association results of *mc4r* genotypes and haplotypes will be presented for the four phenotypes weight, length, height, and body index score. We will also discuss our findings in the light of species-differences in the regulation of energy balance.

#### P09.024

#### Novel extreme homozygote haplotypes at the human Caveolin 1 gene upstream purine complex in sporadic Alzheimer's disease

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Aberrant expression of the caveolin-1 (CAV1) gene is associated with Alzheimer's disease (AD) brain. We report a novel polymorphic purine stretch of GGAA and GAAA motifs located at between 1.8 kb and 1.5 kb flanking the CAV1 gene, whose alleles and genotypes are associated with late-onset AD. Over one hundred haplotypes were detected in the cases and controls as a result of the three polymorphic motifs. Extreme haplotypes were observed in the patients (n=240) that were non-existent in the controls (n=250)(p<0.0006, OR= OR=15.42, CI 2.1-118.2). The overall homozygosity rate for haplotypes was estimated at 0.14 in the AD group versus 0.04 in the controls (p<.0004, OR = 3.68 CI 1.74-7.95). We propose that there is a window for the length of motifs and haplotypes in the controls. Shorter and longer motif and haplotype lengths and homozygosity for those haplotypes were linked with AD in our study. Our findings elucidate novel predisposing haplotypes at the CAV1 gene purine complex, and confirm the role of this region in the etiopathophysiology of late-onset AD. The polymorphic motifs GGAA and GAAA are binding sites for the transcription factor families, Ets and IRF, and are strictly conserved in distantly-related non-human primates, implying likely functionality for those sequences. Remarkably, the interaction of the Ets and IRF family members has been reported in several studies. The effect of this highly complex sequence on the expression of the gene remains to be clarified in the future studies.

#### P09.025

#### Potentially pathogenic networks in celiac disease: long-term and acute effects of gliadin on small intestine of patients

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**Background:** Celiac disease is a complex, immune-mediated intolerance to gliadin that develops in genetically susceptible individuals. Although the main driving force of the disease is an aberrant immune response, several other pathogenic mechanisms must also be involved. In order to describe at a network level the alterations provoked by a gliadin insult on the intestinal mucosa of patients, we compared expression profiling results of biopsies from active and treated patients (long-term effects of gliadin), and of biopsies from gluten-free diet treated patients that were incubated *in vitro* with or without gliadin (acute effects). **Results:** Integration of the 1.647 and 96 significantly altered transcripts identified in the long-term and acute experiments

into potentially pathogenic networks, suggests important dysfunction of processes related to cell-cell communication, intracellular signaling, ubiquitin-proteasome system, cell cycle and apoptosis and extracellular matrix. These genes could be classified into 11 KEGG pathways and associated to 9 Gene Ontology terms related to the networks. **Conclusions:** Our study reconstructs the participation of different biological networks in the development of the intestinal lesion in celiac disease. Comprehensive analysis of expression profiling results at the network level provides a more accurate picture of the events that lead to the disease, and could guide towards novel functional candidates responsible for genetic susceptibility, helping to avoid negative results derived from candidates that were selected on the basis of altered expression levels in single-gene approaches.

#### P09.026

#### Investigation of three candidate genes in the cleft lip and/or palate patients group of Lithuania

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Research of cleft lip and/or palate (CLP) is under thorough investigation in Lithuania for the past decade. There are DNA samples of 140 triads (child with nonsyndromic CLP and both his/her parents) and 250 probands in total collected in the Lithuanian CLP Biobank.

According to the results of the metabolonomial analysis of databases the strongest CLP candidate genes were grouped according to their interplay, to the pathology and to the functional pathways they appear in. Having complemented results of the association studies for five microsatellite markers in TGFA, TGFB3 and BCL3 genes in the Lithuanian group of CLP triads, individuals of 104 Lithuanian CLP triads were genotyped for 50 SNPs within and outside TGFA, 11 SNPs within and outside TGFB3 and 8 SNPs within and outside BCL3 using arrayed primer extension - based genotyping technology on CLP DNA microarray.

Statistical genetic analysis for association testing was performed by using transmission disequilibrium test - ETDT for microsatellite markers analysis and TDT/S-TDT for SNP analysis. But no statistically significant results were found for SNPs in TGFA, TGFB3 and BCL3 genes.

Thus the current results indicate that TGFA, TGFB3 and BCL3 genes are of minor importance to the impact towards the development of cleft lip and/or palate in the population of Lithuania.

The Lithuanian CLP Biobank is constantly being supplemented by new cases and further novel and earlier applied assays are being carried out to investigate the pathogenesis and aetiology of CLP in the population of Lithuania.

#### P09.027

#### Significant association between *IRF6* gene and nonsyndromic cleft lip with or without cleft palate in Latvian population

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**Background:** Cleft lip with or without cleft palate (CLP/CL/CP) is one of the most common birth defects, but its etiology is largely unknown. It is very likely that genetic and environmental factors contribute to this malformation. Mutations in the *IRF6* (interferon regulatory factor 6) gene have been shown to be the cause of Van der Woude syndrome, a dominant disorder that has CLP/CL/CP as a common feature. It has been reported that genetic polymorphisms at the *IRF6* locus are associated with nonsyndromic CLP/CL/CP, with stronger association in several populations.

The aim of the study was to evaluate the relevance of *IRF6* gene in development of nonsyndromic orofacial clefts.

**Materials and methods:** Seven SNPs (rs2073487, rs4844880, rs2013162, rs2235371, rs658860, rs642961, rs861019) in the *IRF6* gene were analyzed with Real-Time PCR technique for allelic association with nonsyndromic CLP/CL/CP in 83 complete case-parent/sib trios and 24 incomplete case-parent trios from Latvia. Observed data were analyzed with transmission disequilibrium test using family based association test (FBAT) software.

**Results:** Significant association between analyzed *IRF6* gene SNPs was found in different study groups. In patients with CLP/CL four of seven SNPs showed significant association with P value < 0.05. CP patient group showed the strongest association with two SNPs (rs658860, rs642961), P value < 0.001.

**Conclusion:** Obtained results showed *IRF6* gene as important contributing factor in CP etiology with some impact on CLP/CL patients what allow us to hypothesize that *IRF6* gene possibly is one of the major gene in the development of isolated cleft palate.

#### P09.028

##### Cleft palate caused by 12q24.33 amplification

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Orofacial clefts are the most frequent craniofacial malformations in humans. Occurrence estimates range between 1/500 and 1/2500 births for cleft lip with or without palate (CL/P) and around 1/2000 births for cleft palate only (CPO). The majority of clefts are isolated, nonsyndromic. The remaining syndromic cases are subdivided into categories on the basis of chromosomal abnormalities, Mendelian single gene syndromes, teratogenic effects and of unknown cause. We performed chromosomal and molecular karyotyping using Affymetrix GeneChip SNP chips on a total of 200 individuals with CL/P or CPO. In one female patient we observed a 3Mb duplication encompassing the region 12q24.3-qter and a 1Mb deletion of the telomeric part of the 22q13.3 region. The girl was the only affected member of the family and presented congenital progressive lymphedema, hypotonia, mental retardation, facial dysmorphism and CPO indicative of a 22q13 deletion syndrome, also known as the Phelan-McDermid syndrome. Interestingly she had CPO, a feature not linked to this well characterized syndrome. We hypothesize that the presence of CP is due to the trisomy 12qter. This locus has not been incriminated in CL/P nor CPO before. This study shows that molecular cytogenetics is a valuable tool for the identification of new genes related to complex diseases.

#### P09.029

##### FAF1 is Associated with Cleft Palate and Pierre Robin Sequence

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Nonsyndromic clefts occur in a wide geographic distribution with an average prevalence of 1/700. Genetic factors involved in cleft lip and palate (CL/P) are thought to be different from those having a role in cleft palate only (CPO). We have recently reported FAF1 as a new gene responsible for CPO and Pierre Robin sequence (PRS). Moreover, we showed that Faf1 is needed for craniofacial development in human, mouse and zebrafish.

In order to replicate our positive association study, we conducted TDTs in an independent series of 160 European families with CL/P. The same FAF1 variant as in our first study was genotyped. In the replication, FAF1 showed positive tendency for association only in the CPO/PRS subgroup (p=0.09). Pooling together our 500 patients reinforced the earlier association, giving a more stringent p-value of 0.001 for the CPO/PRS subgroup. In order to identify other genes contributing to the occurrence of this multifactorial condition, we are testing in parallel association of *IRF6* and *SATB2*, two cleft genes, to the cleft condition in our 500-patient cohort. Preliminary results suggest that, contrary to FAF1, *IRF6* predisposes to CL/P, but not to CPO.

This illustrates the benefit of testing greater number of patients in complex diseases in order to well delineate the true predisposed subgroups. Moreover, it confirms that FAF1 and *IRF6* play a role in the occurrence of isolated complex clefts, but most likely in distinct pathways.

#### P09.030

##### Large CNVs are involved in the pathogenesis of schizophrenia

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We investigated the involvement of rare (< 1%) CNVs in 471 cases of schizophrenia recruited in the UK, and 2792 controls from the UK, used by the Wellcome Trust Case Control Consortium. All samples had been genotyped with the Affymetrix GeneChip 500K Mapping Array. We accepted only CNVs >100kb identified independently on both arrays (Nsp and Sty), with at least 10 SNPs each. Large CNVs >1Mb were 2.26 times more common in cases (p=0.00027), with the effect coming mostly from deletions (OR=4.53, p=0.00013) although duplications were also more common (OR=1.71, p=0.04). Two large deletions were found in two cases each, but in no controls (Fisher Exact Test p=0.02): a deletion at 22q11.2 known to be a susceptibility factor for schizophrenia, and a deletion on 17p12, at 14,0-15,4Mb. The latter is known to cause hereditary neuropathy with liability to pressure palsies (HNPP). Another large deletion affects neurexophilin (*NXPH2*), a gene that interacts with neurexins. *NRXN1* was also disrupted by deletions in one case and three controls (0.2% vs 0.1%, p=0.5).

One large duplication on 16p13.1, between 15,0 and 16,2Mb, was found in three cases and six controls (0.6% vs 0.2%, p=0.13). It has been previously implicated as a susceptibility factor for autism by Ullmann et al (2008). The largest duplication, of 5Mb, was in a schizoaffective case and involved the Prader-Willi/Angelman Syndrome critical region, and is a known susceptibility factor for autism.

This study confirms the involvement of rare and large CNVs in the pathogenesis of schizophrenia.

#### P09.031

##### No association of polymorphisms within the CD44 gene and the coeliac condition

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Coeliac disease is a complex disorder characterised by inflammation, villous atrophy and hyperplasia of the intestinal mucosa in genetically susceptible individuals, upon exposure to dietary gluten. Sequencing of the CD44 gene at locus 11p13-12, revealed a number of sequence variants, one of whom (rs1071695) was linked with an inherited haplotype identified in a previous linkage study.

Three single nucleotide polymorphisms (rs1071695, rs3736812, rs1467558) within the CD44 gene were tested by restriction fragment length polymorphism in a group of coeliac individuals (n=92) and controls (n=248). The mean age at diagnosis for the coeliac group was 34 years, with the predominant presenting symptoms being gastrointestinal.

Genotype frequencies observed for all three polymorphisms studied were in Hardy-Weinberg equilibrium (p>0.05). When testing for linkage disequilibrium (LD) between polymorphisms within the control group the rs1071695 variant in exon 3 was in LD with rs3736812 in exon 4 (p=0.004) but not with rs1467558 in exon 11. The latter, was in LD with rs3736812 (p=0.003). In the coeliac group, LD was only observed between rs3736812 and rs1467558 (p=0.036; Fisher's exact test). No significant association of individual polymorphisms or combined genotypes was observed with the coeliac condition in the studied population. Also no significant association was observed between constructed haplotypes and the coeliac condition ( $\chi^2=4.56$ ; p=0.713; df=7). No association with coeliac was observed between any of the studied SNPs, including the linked variant identified by linkage, in this population based study.

#### P09.032

##### Novel insights into pathogenesis of coeliac disease: a second genome-wide association study

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Coeliac disease (CD) is a common, complex trait with high heritability and strong association to the HLA locus. In the first genome-wide association study (GWAS) we found 10 new loci and pointed to the altered innate and adaptive immunity pathways in CD. There is a remarkable overlap between these loci and those known to be involved in other inflammatory disorders.

We aimed to expand the number of samples and single nucleotide polymorphisms (SNPs) tested in this GWAS. We expanded our cohort to 4,200 cases and 10,500 controls, originating from four European populations and genotyped for the Illumina 550K tag SNP set. We are currently integrating the data and performing a meta-analysis. Validation of the most significant SNPs will be carried out in CD and in samples from patients with inflammatory bowel disease, multiple sclerosis and rheumatoid arthritis. We also extend the analysis of genetic variation from SNPs to common copy number variants (CNVs). We used custom, high-density Illumina Human-670 Quad Genotyping BeadChips, enriched for a novel set of 120,000 CNV probes capturing 5,000 common heritable CNVs. We are now applying different CNV algorithms, including TriTyper and PennCNV.

GWA studies in other autoimmune diseases (e.g. type 1 diabetes, Crohn's disease) have shown that enlarging the sample size enabled many more risk variants to be identified. We expect to double the number of genetic variants and gain novel biological insights into the pathogenesis of CD by discovering further SNP risk variants and, for the first time, performing CNV analysis in coeliac disease.

#### P09.033

##### Several genes on chromosome 4q27 are involved in Coeliac Disease susceptibility

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Association between Coeliac Disease (CD) and SNPs in the 4q27 region, containing the KIAA1109, TENR, IL2 and IL21 genes was detected in British coeliac cases and controls and confirmed in Dutch and Irish collections [van Heel et al. 2007].

To better understand how this region is involved in susceptibility to CD, five SNPs were studied in Italian 407 CD cases and 406 controls. The Combination Test [Jannet et al, 2003] was applied to select the subset of SNPs showing the most significant difference in the genotypic distribution between patients and controls. The best discrimination is obtained through the joint information of 3 SNPs, one located in KIAA1109, the other two on each side of IL21. The range of the relative risks of the 3-SNP genotypes is very impressive, one genotype conferring 9 times more risk than the least at-risk. Our result strongly suggests that several genes in 4q27 are involved in CD susceptibility. The probable role of KIAA1109 and IL21 is also supported by expression studies showing a difference of mRNA levels between CD cases and controls for these two genes.

The importance of the 4q27 region in auto-immunity is well established. The difficulty of identifying causal genotypic variations is illustrated by the problems encountered in disentangling the HLA component of most auto-immune diseases. The modelling of the 4q27 region is likely to also be a long quest, not to mention the added complexity of uncovering its interaction with other genes involved in the pathological pathway(s).

#### P09.034

##### Sp1-binding site polymorphism of COL1A1 gene, juvenile idiopathic arthritis course and bone metabolism

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**Objectives:** To detect an association between the alpha 1 chain of collagen type 1 (COL1A1) gene Sp1-binding site polymorphism and juvenile idiopathic arthritis (JIA) course and bone mineralization in JIA patients. **Study Population:** 192 children with JIA (81 boys and 111 girls). The average age was 11.22±4.43 years.

**Methods:** In all children we checked more than 20 clinical and laboratorial arthritis activity parameters, such as the time of visual activity score (VAS), Ritchie arthicular index (RAI), DAS, DAS28, Shteinbroker index, platelets and leucocytes count, erythrocyte sedimentation rate (ESR). Bone mineralization of lumbar spine (L1-L4) was assessed by dual-energy X-ray absorptiometry with pediatric referral database. About 10 bone metabolic markers, such as total Ca, Ca/P and Ca++/P ratios, total alkaline phosphatase, osteocalcine, C-terminal telopeptides and parathyroid hormone also were determined. Molecular testing of Sp1 COL1A1 polymorphism (rs1800012, +2046G>T) was carried out by polymerase chain reaction followed by Bse11 enzymatic restriction.

**Results:** We did not reveal any differences in polymorphic Sp1 alleles and genotypes distribution between children with normal and low BMD. Girls with GG genotype of Sp1 COL1A1 polymorphism had lower ESR ( $p=0.01$ ), platelets ( $p=0.03$ ), higher Hb ( $p<0.05$ ) and Ca/P ratio ( $p=0.03$ ) compared to girls carrying T allele (GT and TT genotypes). Boys with GG genotype had lower RAI ( $p=0.04$ ), VAS ( $p=0.02$ ) and DAS ( $p=0.04$ ) compared to boys carrying T allele.

**Conclusion:** In our study we have revealed an association between Sp1 polymorphism of the COL1A1 gene and biomarkers of inflammatory activity in children with JIA juvenile idiopathic arthritis.

#### P09.035

##### COL1A1 PCOL2 polymorphism, juvenile idiopathic arthritis course and bone metabolism

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**Objectives:** The aim of our study was to detect an association between the alpha 1 chain of collagen type 1 (COL1A1) gene PCOL2 polymorphism and juvenile idiopathic arthritis (JIA) course and bone mineralization in JIA patients.

**Study Population:** 192 children with JIA (81 boys and 111 girls). The average age was 11.22±4.43 years.

**Methods:** In all children we checked more than 20 clinical and laboratorial arthritis activity parameters, such as visual activity score (VAS), Ritchie arthicular index (RAI), DAS, DAS28, Shteinbroker index, albumin, γ-globulins, platelets and leucocytes count. Bone mineralization of lumbar spine (L1-L4) was determined by dual-energy X-ray absorptiometry with pediatric referral database. About 10 bone metabolic markers, such as total Ca, Ca/P and Ca++/P ratios, total alkaline phosphatase, osteocalcine, C-terminal telopeptides and parathyroid hormone also were assessed. Molecular testing of PCOL2 COL1A1 polymorphism (rs1107946, -1997G>T) was carried out by polymerase chain reaction followed by Psyl enzymatic restriction.

**Results:** We did not reveal any differences in polymorphic PCOL2 alleles and genotypes distribution between children with normal and low BMD. Girls with GG genotype of the PCOL2 COL1A1 polymorphism had lower Ca/P ( $p<0.05$ ) and Ca++/P ratios ( $p<0.05$ ) compare with girls, carrying T allele (GT and TT genotypes). Boys with GG genotype had lower albumin ( $p<0.05$ ) and higher platelets ( $p=0.04$ ), PTH ( $p<0.05$ ) and γ-globulins ( $p=0.03$ ) compared to boys carrying T allele. **Conclusion:** We have revealed an association between PCOL2 polymorphism of the COL1A1 gene and biomarkers of inflammatory activity and bone metabolism in children with juvenile idiopathic arthritis.

## P09.036

## Lack of association between neprilysin (NEP) gene polymorphisms and Complex Regional Pain Syndrome (CRPS) in a German cohort

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Complex Regional Pain Syndrome (CRPS) is a chronic neurological disorder characterized by disabling pain, swelling and impairment of motor function. The disorder usually develops after minor trauma or surgery. Patients with CRPS reveal enhanced sensitivity to experimentally applied substance P (SP), a neuropeptide responsible for neurogenic inflammation. The extended sensitivity is even present at symptom-less stages of the disease. A genetic background for the disease is assumed based on some familial cases and studies describing association of HLA antigens and CRPS. A functional candidate gene approach prompted us to determine whether neprilysin (NEP) as a SP degrading endopeptidase influences disease susceptibility in a German cohort of patients. DNA was obtained from 325 CRPS patients and 376 controls. Initially, we demonstrated association of a GT- repeat polymorphism in the promoter region of NEP with CRPS ( $p < 0.05$ ). To confirm this result, 21 SNPs throughout the NEP gene region were genotyped using allelic discrimination taqman assays. No significant differences in genotype frequencies were observed between controls and CRPS patients. In conclusion, our study did not reveal any association between NEP polymorphisms and CRPS. In addition, our data demonstrate the limitations of candidate gene association studies relying on single associated polymorphisms.

## P09.037

## A high diploid copy number of the beta-defensin CNV associates with severe COPD

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Chronic Obstructive Pulmonary Disease (COPD) is characterized by airflow limitation that is not fully reversible. Smoking is a major environmental risk factor for developing COPD, but not all smokers develop COPD. Linkage studies have shown that the chromosomal 8p23 region, where the defensin repeat region is located, is linked with susceptibility to airflow obstruction. The beta-defensin CNV is polymorphic between individuals and therefore the dosage of these defensin genes/proteins varies.

The aim of this study was to investigate whether the diploid beta-defensin copy number associates with COPD.

We developed a real time PCR assay to quantify the number of beta-defensin repeats in this region. For this purpose we generated 4 concatemeric constructs with 1 copy of *DEFB1* and a particular number of *DEFB4* copies, which ranged from 1 to 4 copies. Using these controls as standards, the number of defensin repeats could be accurately determined in DNA samples.

The diploid beta-defensin copy number was determined in 110 severe COPD patients, 149 age-matched smoking control individuals, and 121 adult control individuals. The severe COPD group had a mean diploid beta-defensin copy number of 5.1, the two control populations had a mean copy number of 4.3. The T-test P-values comparing the COPD patients with the control populations were  $4 \times 10^{-14}$  and  $8.2 \times 10^{-8}$  respectively. The distributions in the two control populations were equal (P-value = 0.8). A strong association between the diploid beta-defensin copy number and the diagnosis of severe COPD was thus found with an Odds ratio of 5 (95% CI 2.57-8.78).

## P09.038

Detection and quantification of intestinal mucosa associated *Escherichia coli* in crohn's disease patients by multiplex real time PCR

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Inflammatory bowel diseases encompasses Crohn's disease and ulcerative colitis, which are exhibited as chronic intestinal inflammation of unknown etiology. Resident bacterial flora plays a pivotal role in the disease development and perpetuation of inflammation. In several recent studies *Escherichia coli* has been suggested as a key feature of Crohn's disease, with increased concentration in the intestinal mucosa tissue. Previous studies, based on plate enumeration had implied a correlation between the amounts of tissue associated *Escherichia coli* and a form or phase of the disease. On the other hand, 16S rDNA gene real-time PCR quantification studies of entire *Enterobacteriaceae* family showed contradictory results, with no change or reduction of enteric bacteria in Crohn's disease tissue. In the present study, we applied multiplex PCR for relative quantification of *Escherichia coli* in the Crohn's disease patients and patients with polyps. A region of 16S rDNA gene was amplified with a single broad range primer pair, targeting *Proteobacteria* with two hydrolysis probes; *Escherichia coli* specific and universal probe for normalization. Normalized values of *Escherichia coli* amount between healthy and inflamed mucosa of Crohn's patients were compared and no statistical significant difference was found ( $p=0.754$ ). Then amount of *Escherichia coli* between Crohn's disease mucosa and healthy mucosa of polyposis patients was compared and significant reduction of *Escherichia coli* was found ( $p=0.04$ ). Finally, amount of *Escherichia coli* between healthy tissue and polyps from polyposis patients showed significant reduction of *Escherichia coli* in polyps as compared with healthy tissue ( $p=0.032$ ).

## P09.039

Analysis of three polymorphisms in *TNF-alpha* gene: c.-857C>T, c.420C>T, c.750A>T, among Polish patients with Crohn's disease.

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Crohn's disease (CD) together with ulcerative colitis (UC) belongs to inflammatory bowel diseases (IBD). Etiology of the CD is still unknown but it is suspected that genetic, environmental and immunological factors play a major role in the background of CD. Currently, the morbidity in the European Union oscillates around 5 new cases per 100,000 people per 1 year and it is still higher.

During last few years some genes of predisposition to CD were localized, including the most significant *locus* - pericentromeric region of chromosome 16 (16p12-q13), called IBD1 and *CARD15/NOD2* gene in this region. Numerous studies showed that polymorphisms in this gene are associated with susceptibility to CD. Other studies implicate regions: 12p13 (IBD2), 6q13 (IBD3), 14q11 (IBD4) or 5q31 (IBD5). One of the genes investigated in the field of Crohn's pathogenesis was also *TNF-alpha* gene.

The aim of this study was to analyze three SNPs within the *TNF-alpha* gene: c.-857C>T, c.420C>T, c.750A>T.

Investigated group consisted of 96 Polish patients with CD and appropriate population group. Genotyping was performed by pyrosequencing. We did not observe any variation among Polish patients with CD and population. We concluded that polymorphisms: c.-857C>T, c.420C>T, c.750A>T cannot be used as markers of Crohn's disease susceptibility in Polish population.

**P09.040****DEFA1/DEFA3 G3400A variation in patients with vesico-ureteric reflux**

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Primary congenital vesico-ureteric reflux (VUR) is a very common urogenital tract disorder. It represents a major risk factor for recurrent urinary tract infections. VUR is considered a complex trait and among possibly many implicated genes may be several that influence infectious agent defense. Therefore it may be plausible to assume an association between VUR and genetic variations in genes with antimicrobial functions. Such genetic variations may not necessarily contribute to the development of VUR but may affect the course of the disease. This study analyzed DEFA1/DEFA3 G3400A variation in the alpha defensin locus for possible association with VUR.

Genotyping was done in 178 children diagnosed with primary congenital vesico-ureteric reflux and in 300 healthy controls with no medical record of reflux. Genomic DNA was extracted from whole blood samples using standard methods. The G3400A variation was detected with PCR-RFLP (HaeIII) using agarose gel electrophoresis.

A statistically significant overrepresentation of patients with DEFA3 3400A variant was observed when compared with the control group. However the frequencies of both variants did not differ significantly between tested groups..

Our results indicate that G3400A variation in the DEFA1/A3 alpha-defensin locus may be associated with VUR. Such variations in alpha defensins may increase the susceptibility of patients with VUR to urinary tract infections and are probably not involved in the development of VUR itself. Further analysis that would include other elements of a complex structured alpha defensin locus are needed to validate a possible role for alpha defensins in patients with VUR.

**P09.041****Changes in gene expression after diabetogenic stimulation analyzed by using DNA microarrays and pathway analysis (GeneGo, MetaCore), a family case report**

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Diabetes mellitus is a disorder of metabolism that manifests itself as type I or type II. The Type I (T1D, the object of this study) is caused by destruction of the insulin-producing beta-islet cells of the pancreas. The aim of our study was to monitor *in vitro* changes in gene expression in peripheral blood mononuclear cells (PBMCs) after stimulation with diabetogenic autoantigens within one family related to different manifestation of the T1D. The situation was very exciting due to family structure which includes identical quadruplets. PBMCs were divided into equal halves and one half was stimulated using derived synthetic peptides. After RNA isolation, amplification and fluorescent labeling, the samples were competitively hybridized on the high density whole-genome microarray HOA (PhalanxBiotech). Hybridized slides were scanned and analyzed using GeneSpring GX (Agilent) and Pathway analysis software MetaCore (GeneGo). The microarray part was done as a service in microarray facility of Central European Biosystems Ltd. We identified genes differentially expressed in each group according to T1D occurrence and family status. In accordance with previously published results we found out the similar expression pattern between basal expression and expression in stimulated samples depending on the diabetes development. It seems that the down or up regulation hit different genes in almost the same metabolic pathways. The study confirms that there can be find differential expression pattern and markers which reveal risk of diabetes.

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**P09.042****Use of a Genetic Isolate to Identify Disease Variants: a new multiple sclerosis locus on 17q21.1**

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Prevalence of multiple sclerosis (MS) in the Southern Ostrobothnia (SO) sub-isolate region of Finland is two-fold compared to other populations of Northern European descent. We used genealogical information reaching up to 15 generations back to construct two regional "megappedigrees". We hypothesize that one or more variants predisposing to MS may be regionally enriched and these pedigrees can be used to identify new MS loci using a genome wide SNP screen.

68 MS cases from Southern Ostrobothnia were genotyped using the Illumina 317K HumanHap panel and 136 IBS matched population samples were used as controls. A five SNP sliding window haplotype analysis on a previously identified 5p12-14 linkage region (Kuokkanen et al. 1996) revealed three associated loci with  $p$ -value  $\leq 10^{-4}$ . One of the regions identified, C7-FLJ40243 locus, showed association in an independent replication set from the SO region ( $p < 10^{-5}$ ) (Kallio et al. 2009).

Genome-wide single SNP association analysis revealed 28 loci with  $p < 10^{-4}$ , including the HLA-locus. The replication of the loci in an independent set of 753 cases and 1029 controls from Finland revealed two associated SNPs outside the HLA locus on 16q21 and 17q21.2, and the 17q locus was further replicated using data available from the meta-analysis of six GWA study sets from 4 populations (2624 cases, 7220 controls from US, UK, Netherlands and Switzerland) ( $p < 10^{-4}$ ) resulting in a combined  $p$ -value of  $p < 5.49 \times 10^{-6}$ . Further validation of the single SNP associations is ongoing in a Nordic MS cohort. This exemplifies the power of population isolate in identifying disease variants predisposing to MS across populations.

**P09.043****Polymorphisms of genes and physical performance in divers**

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The aim of the study was to investigate gene polymorphisms for association with physical performance in Russian elite and sub-elite male divers. Aerobic and anaerobic performance parameters were evaluated by treadmill and Oxycon mobile Gas Analyzer. We analyzed five candidate genes of which PPARA, PPARG and PPARD genes regulate lipid and glucose metabolism of skeletal muscle and heart, VEGF regulates angiogenesis, while mitochondrial DNA replication is under control of TFAM (transcription factor A, mitochondrial). PPARA intron 7 G/C, PPARD +294T/C, PPARG Pro12Ala, TFAM Thr12Ser, VEGF C-2578A gene polymorphisms were determined by PCR-RLFP. We found that PPARA C allele was significantly associated with increased body weight of athletes (GC - 92.3 (7.3) kg, GG - 75.9 (4.6) kg;  $P=0.0045$ ). Furthermore, there was an interrelation between TFAM Thr (Ser/Ser - 569 (43) sec, Ser/Thr - 623 (35) sec, Thr/Thr - 637 sec;  $r=0.71$ ,  $P=0.037$ ) and PPARD C (TT - 568 (42) sec, TC - 628 (27) sec;  $P=0.044$ ) alleles with high values of test working time. Additionally, PPARA G (GG - 53 (1.8) ml/min/kg, GC - 48.5 (3.2) ml/min/kg;  $P=0.032$ ), PPARG Ala (Pro/Pro - 49.2 (3.4) ml/min/kg, Pro/Ala - 52.9 (1.1) ml/min/kg, Ala/Ala - 54.5 ml/min/kg;  $r=0.74$ ,  $P=0.025$ ) and VEGF C (AA - 47.3 (3.8) ml/min/kg, AC - 51.4 (2.7) ml/min/kg, CC - 53.7 (1.1) ml/min/kg;  $r=0.71$ ,  $P=0.037$ ) alleles were correlated with high values of VO2max. In conclusion, PPARA, PPARD, PPARG, TFAM and VEGF gene polymorphisms are associated with physical performance in divers.

**P09.044****Impact of the KDR gene His472Gln polymorphism on endurance-related phenotypes**

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Kinase insert domain receptor (KDR or VEGFR2) is essential to induce the full spectrum of VEGF angiogenic responses to aerobic training. In the present study, we examined the impact of the KDR gene His-472Gln polymorphism on elite athlete status, endurance performance and muscle fibre type composition. Four hundred and seventy one Russian athletes were prospectively stratified into four groups according to event duration, distance and type of activity, covering a spectrum from the more endurance-oriented to the more power-oriented. KDR genotype and allele frequencies were compared to 603 controls. To examine the association between KDR and fibre type composition, vastus lateralis muscle biopsies were obtained from 45 physically active healthy men and 23 all-round skaters. In addition, 76 competitive rowers performed incremental endurance exercise to allow analysis of genotype associations with exercise responses. We found that the frequency of the KDR 472Gln allele was significantly higher in endurance-oriented athletes compared to controls (36.8% vs. 27.4%,  $P = 0.0006$ ). Absolute and relative VO<sub>2</sub>max were significantly greater in the KDR 472Gln allele carriers compared with the His/His homozygotes of male and female rower groups, respectively. Genotype-specific differences were found for the proportion of slow-twitch fibres in both athletes and controls, which was ~10.1% and ~7.4% higher in the His/Gln and Gln/Gln genotypes than in the His/His genotype group, respectively ( $P < 0.05$ ). In conclusion, we have shown that variation in the KDR gene is associated with elite athlete status, endurance performance of athletes and muscle fibre type composition.

#### P09.045

##### Novel progranulin mutations : screening for PGRN mutations in a series of frontotemporal lobar degeneration cases

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Frontotemporal lobar degeneration (FTLD) is a clinically and genetically heterogeneous syndrome. Mutations in two genes, Microtubule-Associated Protein Tau (MAPT) and Progranulin (PGRN) have been linked to this disorder. We looked for PGRN mutations in a series of FTLD patients to evaluate the frequency of PGRN mutations in both sporadic and familial FTLD. Seventeen patients affected by FTLD were subjected to a clinical study and neuroimaging investigations. DNA analysis was carried out to investigate the PGRN gene. In addition, DNA analysis was carried out in 100 healthy neighbor unrelated individuals. We identified three novel pathogenic mutations in the PGRN: a missense mutation in exon 10 (c.1196 A/T, p.Asp399Val), and two different frameshift mutations: p.Gly338GlyfsX22 and p. Val516GlyfsX31, resulting from a nucleotide deletion in exon 9 (c.1014 del G) and a nineteen nucleotide deletion in exon 11 (c. 1547\_1565 del TGAAGGACGTGGAGTGTGG) respectively. The carriers of Asp399Val and Val516GlyfsX31 were affected by frontal variant of FTLD, while the carrier of Gly338GlyfsX22 was affected by a corticobasal syndrome. The carrier of Val516GlyfsX31 had a familial history for dementia, while the other subjects were sporadic. The mutations were absent in all the healthy controls. Moreover, four sequence variations were detected in 11 patients. We disclosed an association between the functional polymorphism 3'UTR +78 C/T at exon 12 and a non-fluent aphasia syndrome, reported in 4 out of the 8 carriers. Three novel pathogenic PGRN mutations were found in 18% of the patients, suggesting a role as major cause of FTLD in our series.

#### P09.046

##### Fragile X premutation alleles in movement disorders

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The fragile X-associated tremor/ataxia syndrome (FXTAS) predominantly occurs in men carrying *FMR1* gene premutation alleles (55-200 CGG rep) over age 50. Many associated symptoms overlap with those

of both Parkinson's disease (PD) and essential tremor (ET). We assayed the *FMR1* premutation genotype in 203 PD patients, 30 ET patients and 370 healthy subjects. We also included two parkinsonian patients from a fragile X mental retardation pedigree, and two cases with intention tremor and postprandial hypotension. All participants had the same ethnic background and gave informed consent. We did not find *FMR1* premutation genotype in any patients with PD and ET or in any healthy controls. There were 17 distinct alleles, ranging from 19 to 37 CGG repeats. On the contrary, the two subjects with parkinsonian symptoms and family history of fragile X syndrome carried, respectively, 57 and 90 CGG repeats. Concerning the two subjects with intention tremor and postprandial hypotension, one of them had 73 CGG repeats and the second one was an uncommon mosaic for a premutation (90 CGG rep) and a normal-size allele. The wide and variable FXTAS phenotype overlaps the clinical features of many neurological diseases, making diagnosis of this disorder difficult without molecular analysis. Our data show that premutated alleles are rare in PD as well as in ET. Thus the presence of postprandial hypotension or a positive family history of fragile X syndrome, beside the peculiar T2-hyperintense signal in middle cerebellar peduncles, can be considered an important indication for *FMR1* expansion genetic testing.

#### P09.047

##### ESR1, LPL and APO E gene variants in relation to lipid status and obesity in young healthy subjects

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Background. Human obesity is a multifactorial syndrome influenced by both environmental and genetic factors. Among gene variants found to be involved in body weight regulation and development of obesity, particular attention has been paid to polymorphisms in genes related to adipogenesis, energy expenditure, and insulin resistance. We explored the association of genetic polymorphisms of: PPARG2, Pro12Ala; adiponectin (ADIPOQ -11391G>A and 11377C>G); IL-6-174G>C; estrogen receptor (ESR1alfa-TA); APOE; ACE (I/D); MTHFR-677C>T; LPL (PvuII+/-), with clinical variables: gender, age, BMI, and biological variables: triglycerides, cholesterol, HDL, LDL, CRP, homocysteine, glucose, in 105 healthy young subjects, (20-35 y) of Croatian origin.

Methods. Genotyping of PPARG2, IL-6, ACE, LPL was performed by PCR-RFLP, APOE, MTHFR, ADIPOQ, by real-time PCR and ESR1alfa by capillary electrophoresis. Associations were performed of alleles, genotypes and haplotypes with biological variables.

Results. BMI was increased ( $>25$ ) in 23% of subjects. Increased cholesterol values ( $>5.0$  mmol/L) were found in 23% of subjects, LDL ( $>3.0$  mmol/L) in 23%, triglycerides ( $>1.7$  mmol/L) in 11.4% of subjects. We found statistically significant differences in subjects' weight ( $p=0.015$ ), BMI ( $p=0.023$ ), and hip/waist ratio ( $p=0.015$ ) in regard to their diet type; subjects with Mediterranean diet had the lowest values compared to continental and mixed diet. Significant associations were found for: LPL (PvuII+) genetic polymorphic variants and abdominal obesity ( $p=0.018$ ); APOE4 variant and high LDL ( $p=0.0017$ ); ESR1-L allele and hipercholesterolemia ( $p=0.023$ ).

Conclusions. LPL, APO E and ESR1 genetic polymorphic variants represent predictive genetic risk markers for lipid status and obesity in young healthy subjects.

#### P09.048

##### A genome wide analysis identifies genetic variants in the RELN gene associated with otosclerosis

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Otosclerosis is a common form of hearing loss characterized by abnormal bone remodelling in the otic capsule. The etiology of the disease is largely unknown, and both environmental and genetic factors have been implicated. To identify genetic factors involved in otosclerosis,

we used a case-control discovery group (604 samples) to complete a genome-wide association (GWA) study with 555,000 single nucleotide polymorphisms (SNPs) utilizing pooled DNA samples. By individual genotyping of the top 250 SNPs in a stepwise strategy, we identified two highly associated SNPs that replicated in two additional independent populations (replication set1: 784 samples; replication set2: 935 samples). We then genotyped 79 tagSNPs to fine map the genomic regions defined by the associated SNPs. The region with the strongest association signal ( $p_{\text{combined}}=6.23 \times 10^{-10}$ ; OR:1.52), is on chromosome 7q22.1 and is located in the gene Reelin (RELN), a gene known for its role in neuronal migration. Evidence for allelic heterogeneity was found in this region. We confirmed expression of RELN in the inner ear and in stapes footplate specimens. Consequently, we genotyped 7 SNPs in this region in 4 additional small European populations (1095 samples total). Several SNPs replicated in the populations separately. However, the power of these populations is small, but when combining all 4 populations, 6 of the 7 SNPs replicated again in the same direction as in the previous populations ( $p_{\text{combined}}=5.25 \times 10^{-6}$ ). In conclusion, we provide evidence that implicate RELN in the pathogenesis of otosclerosis. These results point towards a possible new function for RELN in bone metabolism.

#### P09.049

#### Headache epidemiological study: do different diagnosis criteria make a difference?

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Headache is a common disorder and a public health problem. Genetics of headaches (especially in migraine's field) had some improvements in last years, but is still difficult to establish genotype-phenotype correlations. A major issue is thus the correct definition of the phenotype. The "International Headache Society" classification published in 1988 led to a major development headaches' diagnosis. Its revision in 2004 aimed to improve clinical diagnosis however, its consequence on epidemiological data is so far unknown.

We assessed if the application of the new criteria to data from a previous headache epidemiological study (from 1995) changed significantly headaches' diagnosis.

The chance-corrected agreement rate (Cohen's Kappa) was used to compare the ICHD-I and ICHD-II criteria and to evaluate the agreement between the two classifications regarding 1) primary and combined headaches, and 2) frequency of migraine and tension-type headache in the population.

Lifetime prevalence of headaches was 88.6% (1780/2008 individuals). Applying ICHD-II (2004) criteria, 84.7% had primary headaches (80.4% in ICHD-I (1988)) whereas 6.0% had secondary headaches (6.0%) and 9.3% combined headaches (13.6%).

We found a chance-corrected agreement rate of 78% for primary and combined headaches, of 91% for frequency of migraine and of 95% for frequency of tension-type headache ( $P<0.001$ ).

Regarding clinical implications, ICHD-II, by creating new subgroups, allowed the decrease of the uncertainty of probable headache diagnosis.

The ICHD-II criteria may have relevant clinical implications but do not change remarkably epidemiological results. These are important to obtain accurate headache diagnosis in further epidemiological studies and to perform genotype-phenotype correlations.

#### P09.050

#### Analysis of cytokine polymorphisms in a patient with Hereditary Sclerosing Poikiloderma of Weary

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Hereditary sclerosing poikiloderma of Weary (HSP) is a rare syndrome characterized by generalized poikiloderma with accentuation in flexural regions, sclerosis of palms and soles, linear hyperkeratotic and sclerotic bands in the axillae and antecubital and popliteal fossae, clubbing of the fingers, and tissue calcinosis as a late manifestation in one patient. This report presents a patient with HSP, suffering from

difficulty in mastication and speech, together with limited lip closure related to the clinical outcomes of syndrome.

The aim of reporting this case is to detail the first widely determined periodontal abnormalities of a rare syndrome and in DNA by investigating cytokine and MIF (-173) genotype.

Genomic DNA was extracted from mononuclear cells obtained from EDTA-treated peripheral venous blood using the salting out method techniques. Cytokine (IL-6, IL-10, IFN- $\gamma$ , TGF- $\beta$ 1, TNF $\alpha$ ) genotyping was performed by the polymerase chain reaction sequence-specific primer method. The MIF genotyping was determined by polymerase chain reaction-restriction fragment length polymorphism method.

These results are the first detailed genetic study data pertaining to HSP in literature. The TGF $\beta$ 1 (codons 10 and 25) and IL-6 (-174) polymorphisms were detected as high expression while TNF $\alpha$  (-308, -238, -857) was detected as low expression. In addition, the IL-10 (-592, -819, -1082) and INF $\gamma$  (+874) were intermediate expressions and GC genotype in the MIF (-173) gene.

#### P09.051

#### Heroin dependence is associated with dopamine D2 receptor TaqI polymorphisms

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Heroin dependence is a serious social and public health problem. Substance misuse is thought to be influenced by multiple genetic and environmental factors. The genetic predisposition presumably consists of several genetic risk factors with small individual effect. The dopaminergic pathway is a part of the brain reward system, therefore the dopamine receptors are the main candidate genes of addiction. Our aim was to determine specific dopaminergic risk and/or protective factors in the background of heroin addiction.

In our case-control study 300 heroin dependent subjects and 555 sex-matched healthy Caucasian (Hungarian) individuals were genotyped for polymorphisms of the dopamine D2 receptor (DRD2), dopamine D4 receptor (DRD4), dopamine transporter, and the catechol-o-methyl transferase genes.

The TaqI polymorphisms of the DRD2 gene were associated with heroin dependence. The minor A1-allele of the TaqIA polymorphism was increased ( $p = 0.0014$ ) and the A1A1 genotype was two times more frequent ( $p = 0.0076$ ) among the cases. Also the minor B1 (A-allele) of TaqIB polymorphism was associated with heroin dependence: both B1-allele and B1B1 and B1B2 genotypes were overrepresented among heroin users ( $p = 0.0007$ ,  $p = 0.0036$ , respectively). However, the TaqID allele- and genotype-frequencies were not significantly different between the case and the control groups.

Our results indicate that the DRD2 TaqIA and TaqIB polymorphisms contribute to the genetic risk of heroin dependence in Hungarian individuals. These results also support the widely accepted notion about the involvement of DRD2 in substance misuse.

#### P09.052

#### Identification of novel proteins interacting with the RET9 receptor using the "Split-Ubiquitin Yeast Two Hybrid System"

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The proto-oncogene RET encodes a transmembrane receptor tyrosine primarily expressed in neural crest-derived and urogenital cells. Two main isoforms of RET are generated by alternative 3' splicing: the long and short RET isoforms (RET51 and RET9), which differ by 51 and 9 amino acids in the C-terminus. RET51- and RET9-associated signaling complexes are markedly different, suggesting that distinct isoforms can exert different physiological functions.

Loss-of-function mutations of RET have been associated with Hirschsprung disease, a developmental enteric nervous system defect. Mutations and rearrangements with a gain-of-function effect result in thyroid cancer (Multiple Endocrine Neoplasia Type 2A-MEN2A- and 2B-MEN2B-, Familial Medullary Thyroid Carcinoma-FMTC- and Papillary Thyroid Carcinoma- PTC-).

To shed light on the molecular mechanisms of RET9-associated diseases, we performed a screening to identify the interacting proteins of RET9, using a modified two-hybrid yeast complementation assay, the

“Split-Ubiquitin Yeast Two Hybrid System”.

The approach of the “Split-Ubiquitin Yeast Two Hybrid System” is a novel method capable to identify interactions between transmembrane proteins or between a transmembrane protein and a cytoplasmic protein. This method allowed us to identify ten proteins potentially interacting with RET9.

After confirming these interactions by co-immunoprecipitation assay in mammalian cell lines, the pathways involving RET9 and these proteins will be studied. Interestingly, these genes might be considered as good candidates as modifier genes in the pathogenesis of RET9-associated diseases.

**P09.053**

**Study of genetic predisposition to severe course of IBD**

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Inflammatory bowel disease (IBD) is characterized by chronic relapsing inflammation in gastrointestinal tract. This autoimmune disease is divided into two main subtypes Crohn disease (CD) and ulcerative colitis (UC). The genetic bases of predispositions have been still studied. In our 160 severe IBD patients with average age of diagnosis 26 years, the youngest patient was diagnosed when was 3 years old and the oldest one was diagnosed at the age of 69. In this group we investigated frequency of alleles in NOD2/CARD15 gene and 15-PGHD gene. The 15-PGHD gene codes dehydrogenase which is a prostaglandin-degrading enzyme and acts as an antagonist to enzyme called cyclooxygenase 2. We also studied frequency of haplotype in q31 region on 5th chromosome. We estimated frequency of alleles SLC22A4 1672T and SLC22A5 / T207C. In studied group we observed increased frequency of INV4+39C>T homozygotes in group of patient under 18 years old with UC (12%) in comparison to adult patients where the INV4+39C>T homozygotes were not been observed. The frequency of A at position 168 in PGHD gene were higher in patient under 18 years old (49%) than in adult patients 34%. In Nod2 gene we observed statistically significant differences of frequency of P268S, R702W and 2030insC in group of patient with severe course of IBD in comparison to unselected IBD patients and control group

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**P09.054**

**Association of FCER1A and RAD50 polymorphisms with serum IgE levels, asthma and rhinitis.**

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Recently genetic polymorphisms in the gene encoding the alpha chain of the high affinity receptor for IgE (FCER1A), in the RAD50 gene, and in STAT6 gene, were associated with total serum IgE levels (Weidinger et al, PLoS Genet. 2008; 4:e1000166).

In order to assess these results in the Italian population, the association of the most significant reported SNPs (rs2511211, rs2427837 in FCER1A gene; rs2706347, rs3798135, rs2040704, rs7737470 in RAD50 gene, and rs12368672, rs11172106, rs12309413 in STAT6 gene) with serum IgE levels asthma, bronchial hyper-responsiveness, skin prick test, and rhinitis was investigated.

211 Italian families ascertained through an allergic asthmatic child were analyzed.

Single SNP analysis was computed using the computer program sibling disequilibrium test (SDT).

IgE levels were significantly associated with rs2511211 of FCER1A gene ( $p=0.02$ ), and with rs2706347 and rs2040704 of RAD50 gene ( $p=0.01$ ,  $p=0.03$  respectively). Moreover, rs2511211 in FCER1A was associated also with rhinitis ( $p=0.007$ ), and rs7737470 in RAD50 with asthma ( $p=0.01$ ). None of the STA6 analysed SNPs showed significant results. In conclusion, the association of FCER1A and RAD50 gene polymorphisms and total serum IgE levels was confirmed in a different population, and it was extended to asthma and rhinitis phenotypes.

**P09.055**

**GWAS in Sardinians reveals novel loci for levels of inflammatory biomarkers**

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Inflammatory biomarkers, including Interleukin-6 (IL-6), C-reactive protein (CRP), Monocyte chemotactic protein-1 (MCP-1), are primarily produced in response to tissue injury, infection or inflammation during both acute phase and chronic inflammatory processes. Serum levels of these markers as well as values of erythrocyte sedimentation rate (ESR) are used for the diagnosis and management of different inflammatory conditions, and provide insights into atherosclerosis, a chronic inflammatory disease associated with cardiovascular risk, which is the leading cause of morbidity and mortality in western countries. Identifying the genes influencing inflammatory biomarkers could help understanding the genetic determinants of cardiovascular disease (CVD) and predicting individual CVD risk. To identify genetic factors for circulating levels of inflammatory biomarkers we have conducted a genome-wide association scan (GWAS) in 4,305 Sardinian volunteers from the SardiNIA project. Using 356,359 autosomal SNPs that passed quality controls, we imputed an additional ~2 million SNPs using the HapMap CEU haplotypes as reference panel. We evaluated the additive effect of imputed and genotyped SNPs using a family-based association test, adjusting the model for covariates. GWAS confirmed a previous locus for CRP (CRP gene) and revealed new loci associated with serum IL-6, MCP-1 levels and measures of ESR above genome-wide significance threshold ( $p=5\times 10^{-8}$ ). We confirmed association of top markers in an independent sample of 1862 Sardinians (combined p-values for IL6  $<1\times 10^{-22}$ , CRP  $<1\times 10^{-17}$ , MCP-1  $<1\times 10^{-52}$  and ESR  $p<1\times 10^{-13}$ ). Replication in 1200 individuals from the InChianti and 800 from the BLSA studies is ongoing. Preliminary data support our initial findings for associated SNPs.

**P09.056**

**Replication of Signals from Recent Inflammatory Bowel Disease Genome Wide Scans in a Lithuanian cohort**

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**Introduction:** Inflammatory bowel disease (IBD), ulcerative colitis (UC) or Crohn disease (CD), are chronic gastrointestinal inflammatory disorders with a complex genetic background. The number genetic variations conferring to IBD susceptibility increased over the past few years after the introduction of genome-wide association scans (GWAS).

**Aim:** To perform a comprehensive association analysis of genetic markers reported by the two previous GWAS studies [1,2] to further characterize the CD and UC associations in a Lithuanian case-control sample set.

**Material And Methods:** A set of 43 SNPs from three different categories were selected: (1) 6 SNPs reported by Rioux et al.; (2) 12 SNPs - 'non-converging' CD markers and (3) 25 SNPs -'converging' CD markers reported by WTCCC and replicated by Parkes et.al.. They were genotyped in a cohort of 152 UC, 73 CD patients and 249 unrelated healthy controls using SNplex genotyping technology (Applied Biosystems, USA). Assessment of all SNPs and single-marker association analysis were performed using the program Haplovie 4.0.

**Results:** Single-marker analysis revealed marginal associations between IBD and genetic variants from category (2) and (3): CD and rs17419032 (locus: 1q32.1;  $p=4.96\times 10^{-2}$ ) and rs9993022 (locus: 4q13.1;  $p=4.78\times 10^{-2}$ ); UC and rs10883365 (gene: NKX2-3;  $p=2.67\times 10^{-2}$ ), rs17419032 (locus: 1q32.1;  $p=3.44\times 10^{-2}$ ), rs12529198 (gene: LYRM4;  $p=3.22\times 10^{-2}$ , OR=0.37 (95% C.I.: 0.15-0.93)) and rs9895062

(gene: STX8;  $p=9.64\times 10^{-3}$ , OR=0.34 (95% C.I.: 0.16-0.78)).

Conclusion: We replicated genetic associations for CD with 4q13.1, UC with NKKX2-3, LYRM4, STX8, both subtypes with 1q32.1.

References:

1. Rioux, J.D. et al. *Nat. Genet.* 39, 596-604 (2007).
2. Parkes, M. et al. *Nat. Genet.* 39, 830-832 (2007).

#### P09.057

##### Clinical significance of NOD2/CARD15 and TLR 4 gene SNPs in inflammatory bowel disease.

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Background: To evaluate the role of genetic factors in the pathogenesis of Crohn's disease (CD) and ulcerative colitis (UC), we investigated the single nucleotide polymorphisms (SNPs) of NOD2/CARD15 (R702W, G908R and L1007finsC), and Toll-like receptor 4 (TLR4) genes (D299G and T399I) in a selected inflammatory bowel disease (IBD) population coming from Southern Italy.

Methods: Allele and genotype frequencies of NOD2/CARD15 (R702W, G908R and L1007finsC) and TLR4 (D299G and T399I) SNPs were examined in 133 CD patients, in 45 UC patients, and in 103 healthy controls.

Results: NOD2/CARD15 R702W mutation was significantly more frequent in CD (9.8%) than in controls (2.4%,  $P = 0.001$ ) and in UC (2.3%,  $P = 0.03$ ). No significant difference was found between UC patients and control group ( $P > 0.05$ ). In CD and UC patients, no significant association with G908R variant was found L1007finsC SNP showed an association with CD (9.8%)

compared with controls (2.9%,  $P = 0.002$ ) and UC patients (2.3%,  $P = 0.01$ ). Moreover, in CD patients, G908R and L1007finsC mutations were significantly associated with different phenotypes compared to CD wild-type patients. No association of IBD with the TLR4 SNPs was found in either cohort (allele frequencies: D299G-controls 3.9%, CD 3.7%, UC 3.4%,  $P > 0.05$ ; T399I-controls 2.9%, CD 3.0%, UC 3.4%,  $P > 0.05$ ).

Conclusion: These findings confirm that, in our IBD patients selected from Southern Italy, the NOD2/CARD15, but not TLR4 SNPs, are associated with increased risk of CD.

#### P09.058

##### High resolution melting curve analysis for high-throughput SNP genotyping in IL23R and NOD2/CARD15 genes

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Single nucleotide polymorphism (SNP) analysis is important tool in the studies of genetic factors associated with complex diseases and genetically influenced response to drug therapy (pharmacogenetics). We developed a HRM method for NOD2 (rs2066845, rs2066844, rs2066847) and IL23R (rs7517847) genes, associated with inflammatory bowel diseases (IBD). In this study, we demonstrate, that HRM is simple, fast and reliable method (95% confidence) for genotyping clinical samples. HRM analysis is an efficient method for SNP detection and/or genotyping, where homozygotes (GG and TT) were determined with »Tm calling method« differed by 0.53°C for IL23R gene and by 0.1°C, 0.61°C in 0.45°C for NOD2 gene SNPs rs2066845, rs2066844, rs2066847, respectively. Difference between homozygotes and heterozygotes was easily distinguishable by different melting curve shapes with »gene scanning method«.

Additionally, we genotyped 345 Slovenian healthy controls and 295 IBD patients including 195 with Crohn's disease (CD) and 136 with ulcerative colitis (UC) for rs7517847 polymorphism in IL23R gene using standard RFLP and optimized HRM methods. We found strong statistically significant association of IL23R polymorphism with Slovenian CD patients. Allele frequency of minor allele G was 0.46 in controls and was reduced to 0.33 in CD patients ( $p < 0.001$ , OR=0.588). The frequency of G/G genotype carriers was lower in CD patients (8.2%) than in controls (18.6%,  $p=0.002$ , OR=2.558). We found slightly less significant association between IL23R polymorphism and Slovenian UC patients. Carriers of T/T genotype have higher risk for UC ( $p=$

0.035, OR=1.599). This results suggest IL23R plays important role in IBD pathogenesis in Slovenian patients.

#### P09.059

##### Expression patterns and biochemical abnormalities in the insulin signaling pathway components in girls with hyperandrogenism and insulin resistance

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Background: Previous studies in adults report that insulin signaling abnormalities in the muscle of patients with hyperandrogenism can predict glucose intolerance and type 2 diabetes. The aim of the current study was to describe these abnormalities in girls and adolescents with hyperandrogenic states.

Methods: We analyzed girls with clinical/biochemical hyperandrogenism. Patients were classified as (1) hyperandrogenic (N=7) if they had a history of precocious pubarche or hirsutism and biochemical hyperandrogenism, and (2) those with Polycystic Ovary Syndrome (PCOS) (N=13) according to Rotterdam criteria. Analysis of anthropometric (Body mass index [BMI], Waist circumference [WC]) and laboratory data (fasting insulin, OGTT, lipid profile, blood pressure) were analyzed. Muscle biopsies were obtained from vastus lateralis and IRS-1, IRS-2. GLUT-4 expression was measured by RT-PCR in vivo and after primary myotubes cultures. Biopsies from non-obese non-hyperandrogenic and metabolic stable adolescents were used as controls (N=6).

Results: IRS-1 and IRS-2 expression was significantly different in cases versus controls ( $p < 0.01$ ). GLUT-4 expression did not show differences between the groups. HDL-cholesterol was significantly lower in hyperandrogenic compared to PCOS girls ( $p < 0.05$ ). There were no significant differences between both hyperandrogenism groups in the prevalence of insulin resistance indexes and metabolic syndrome.

Conclusions: Overexpression of IRS-1 and IRS-2 was persistently detected in the overall sample of hyperandrogenic girls compared to controls. Impaired insulin action involves posttranslational modification of signaling molecules, most likely including altered phosphorylation of IRS-1 and IRS-2 substrates.

#### P09.060

##### ABCB4 mutations or rare variants are identified in intrahepatic cholestasis of pregnancy (ICP) with small differences in patients with high and normal serum levels of gamma-glutamyltranspeptidase (γGT) activity

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Intrahepatic cholestasis of pregnancy (ICP) is a liver disorder with multifactorial etiology that appears during late pregnancy; it is characterized by maternal pruritus and by biochemical laboratory abnormalities, including normal or high serum gamma-glutamyltranspeptidase (γGT) levels. Within 2 to 3 weeks after delivery there is a spontaneous relief of signs and symptoms but ICP is associated with increased fetal distress, preterm delivery and stillbirth occurring near term. The genetic aetiology of ICP is heterogeneous because heterozygous mutations in at least three different genes encoding proteins involved in bile formation have been described. Although serum γGT levels have been considered a marker to differentiate between ICP with high γGT due to ABCB4 gene mutations and ICP with normal γGT caused by mutations in others two genes (ATP8B1 and ABCB11), a few studies also have reported ABCB4 mutations in ICP women with normal γGT values. Between August 2005 and December 2008, we enrolled 72 women with ICP phenotype (10 with high γGT serum levels) and all the 27 coding exons of ABCB4 gene were sequenced. In thirteen patients (18%), 2/10 with high γGT and 11/62 with normal γGT, we identified 15 heterozygous mutations or rare variants: 9 cause single amino acid change, 4 cause plausibly mRNA misprocessing and 1 causes truncated pro-

tein. Our finding indicate that ABCB4 mutations seem to predispose to ICP phenotype in less than 20% of the affected women, irrespective of serum  $\gamma$ GT levels.

#### P09.061

##### Variation in the interleukin-1 receptor-associated kinase 3 gene and susceptibility to sepsis induced-acute lung injury

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Sepsis is the most common cause of acute lung injury (ALI), organ dysfunction and death in critically ill patients. The Toll/NF- $\kappa$ B signaling pathway has a key role in the immune response to infections. Variants in two genes encoding for interleukin-1 receptor-associated kinases (IRAKs) modify the immune response to pathogens and associate with increased risk for severe complications during sepsis. Based on gene expression data, showing up-regulation of *IRAK3* in both animal models of sepsis and in septic patients, here we explored whether common variants of this gene were associated with susceptibility and outcomes in severe sepsis. We re-sequenced 23 kb non-repetitive regions of the gene in 32 Spanish samples, and selected a set of 7 tagging SNPs (tagSNPs) that efficiently captured common variation in the population. To test the association, tagSNPs were genotyped in 214 severe sepsis cases and 336 population-based controls using the MassArray® iPLEX Gold (Sequenom Inc.). One tagSNP showed a significant association with ALI ( $p=0.005$ ), and remained significant after multiple testing adjustments. Indirect testing of untyped alleles revealed two correlated SNPs ( $r^2=0.85$ ) from the 5' flanking region of the gene associated with ALI (permuted  $p=0.02$ ), that were validated by further genotyping and direct testing ( $p<0.05$ ). A TRANSFAC-based exploration predicted one of them (-1464A/G, MAF=0.25) to be located within a human-mouse conserved FOXP3 transcription factor binding site. This supports that common variation in *IRAK3* gene might be a novel determinant of severe sepsis outcomes.

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#### P09.062

##### Genome-wide association study of iron parameters

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Serum iron parameters indicate the availability of and/or the demand for iron in the body. Heritability measures of these parameters range between 23% (Fe) and 66% (transferrin; Whitfield et al 2000). In case of serum transferrin, 40% of the genetic variance has been attributed to variants in the transferrin gene *TF* and the hemochromatosis gene *HFE* (Benyamin et al 2009). In order to identify further genetic variants of iron homeostasis we determined serum iron parameters (Fe, ferritin, transferrin, soluble transferrin receptor, ferritin index, transferrin saturation) in two population samples from Southern Germany (KORA-gen). These samples comprise 1644 and 1953 subjects from the general population that have been genotyped on Affymetrix 500K and 6.0 platforms, respectively. A genome-wide association study (GWAS; linear regression on allele dosage with age and sex as covariates) reproduced the association of iron parameters with the *HFE* variant C282Y. The highest significance was obtained for the transferrin saturation phenotype. Analyzing transferrin also reproduced the known as-

sociation with *TF* variants. In addition, a strong association between a locus on chromosome 11 and the soluble transferrin receptor was found. Further association signals were detected that had p-values in the range of  $10^{-7}$ . Replication analysis which is currently ongoing will show whether these signals can be confirmed in independent population samples.

#### P09.063

##### A coding variant in the serotonin receptor 3C subunit is associated with diarrhea-predominant irritable bowel syndrome

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Serotonin type 3 (5-HT<sub>3</sub>) receptor antagonists are beneficial in some but not all patients with diarrhea-predominant irritable bowel syndrome (IBS-D). As we recently found variants of the 5-HT<sub>3</sub> subunit genes *HTR3A* and *HTR3E* to be associated with IBS-D, the aims of this study were to investigate whether variants of the *HTR3C* subunit gene may also contribute to the IBS phenotype and to perform pharmacological analyses to provide insight into the functional consequences of respective variants.

*HTR3C* genotyping in a pilot study cohort of 197 IBS and 100 healthy subjects revealed the c.489C/c.489C genotype of the *HTR3C* c.489C>A (rs6766410, p.N163K) coding variant to be associated with female IBS-D ( $P=0.0019$ ; OR = 4.98 CI = [1.75-14.16]), whereas no association could be found in males. Calcium influx analyses of the 5-HT<sub>3A/C</sub> p.163N and 5-HT<sub>3A/C</sub> p.163K receptors resembling the homozygous genotypes revealed identical potencies of 5-HT and two 5-HT<sub>3</sub> antagonists. However, 5-HT showed decreased efficacy at 5-HT<sub>3A/C</sub> p.163N (78.1 ± 5.9 %) compared with 5-HT<sub>3A/C</sub> p.163K (100 %,  $P<0.01$ ,  $n=14$ ) receptors and radioligand binding assays revealed a reduced  $B_{max}$  for the 5-HT<sub>3A/C</sub> p.163N (86.2 ± 3.7 %) compared with the 5-HT<sub>3A/C</sub> p.163K receptor (100 %,  $P<0.05$ ,  $n=6$ ). The decreased 5-HT<sub>3A/C</sub> p.163N 5-HT maximum response, most likely caused by reduced cell surface expression of the mature receptor in comparison to the 5-HT<sub>3A/C</sub> p.163K receptor, may result in altered 5-HT<sub>3</sub> receptor signal transduction in the enteric and central nervous system and thereby contribute to the pathophysiology of IBS-D.

#### P09.064

##### An acquired JAK2 V617F mutation in patients with hypercoagulability disorder

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The pathogenesis of venous thrombosis is multifactorial, involving acquired and genetic factors. Recently described acquired gain-of-function mutation in the JAK2 gene causing dysregulated proliferation of hematopoietic precursors that results in increased number of peripheral blood cells, the major feature of polycythemia vera and essential thrombocythemia, and has been identified as a potential risk factor for thromboembolic events. We therefore investigated whether the prevalence of the JAK2 V617F mutation vary significantly in different clinical groups of patients with venous thrombosis.

Samples of genomic DNA were extracted from peripheral venous blood. An allele specific PCR and agarose gel electrophoresis were used for the detection of the JAK2 V617F mutation. Specific discrimination of the JAK2 V617F allele was achieved by using locked nucleic acid (LNA) nucleotide incorporated in one primer to obtain sufficient allele specificity of amplification.

We collected 508 patients with hypercoagulability disorder and 5000 apparently healthy individuals as the controls. Among patients with hypercoagulability disorder, eight (1.6 %) carried the JAK2 V617F mutation. Five of them had diagnosed deep venous thrombosis of lower extremity or extremities, two patients had cerebrovascular accidents, and one had pulmonary embolism. Five (0.1 %) members of a control group carried the JAK2 V617F mutant allele.

We have found statistically significant higher prevalence of the JAK2 V617F mutation among the patients with hypercoagulability disorder in comparison with a control group. The presence of the JAK2 V617F may represent a potential risk factor for the development of venous thrombosis.

## P09.065

**A Study of Kashin-Beck Disease in the homogeneous population of Tibet in China**R. S. Gunasekera<sup>1,2</sup>, J. Cokenour<sup>1</sup>, P. Sen<sup>3</sup>, D. Heath<sup>4</sup>, M. Han<sup>5</sup>;<sup>1</sup>University of Houston-Victoria, Victoria, TX, United States, <sup>2</sup>Humanitarian Solutions, Inc., Pearland, TX, United States, <sup>3</sup>Baylor College of Medicine, Houston, TX, United States, <sup>4</sup>Innovative Humanitarian Solutions, Inc., Pearland, TX, United States, <sup>5</sup>ProHealth Physicians, Manchester, CT, United States.

Kashin-Beck disease (KBD) is an osteoarthropathy which manifests in children marked by dramatically low levels of serum selenium, iodine, bone and joint deformity, and limited mobility. The disease is endemic to rural Tibet, China; North Korea, and Siberia. Early investigations had led to the hypothesis that the disease was due to dietary factors, due to deficiencies in certain essential nutrients in high plateaus. However, supplementation of the deficient trace minerals conducted by others has had no positive effect on affected persons. Our group has begun investigations to the possibility that genetic elements may also be involved with possible environmental effects *in-utero*. Pedigree studies were conducted on 200 individuals in nuclear families with clinical symptoms. Patients observed ranged from 6 to more than 60 years. KBD was diagnosed when an affected person had persistent pain, restricted mobility, or deformity of the knees, ankles, elbows, wrist, interphalangeal joints, hips, or shoulders, in the absence of trauma. Preliminary analysis suggests KBD having an autosomal recessive pattern of inheritance in most families with a possible higher penetrance in women. Occurrence of the disease exhibits familial aggregation while suggesting the form of inheritance polygenic, and due to multifactorial factors. Initial studies further suggests that deficiencies in selenium and iodine may not be causal, but markers of an underlying condition of extreme oxidative stress brought on by reactive oxygen species acting to inhibit proper mesenchymal cell and bone development by apoptosis. This study attempts to describe pedigree investigations and nutritional genomics of the disease.

## P09.066

**Association of the ACE and BDKRB2 gene polymorphisms with physical performance of kayakers**

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Circulating angiotensin I converting enzyme (ACE) exerts a tonic regulatory function in circulatory homeostasis, through the synthesis of vasoconstrictor angiotensin II, which also drives aldosterone synthesis, and the degradation of vasodilator kinins. A polymorphism in intron 16 of the human ACE gene has been identified in which the presence (I allele) rather than the absence (D allele) of a 287 bp Alu-sequence insertion fragment is associated with lower serum and tissue ACE activity. Bradykinin is a potent endothelium-dependent vasodilator and acts via the bradykinin B2 receptor (encoded by BDKRB2). The absence (-9), rather than the presence (+9), of a 9 bp repeat sequence in exon 1 has previously been shown to be associated with increased gene transcription and higher BDKRB2 mRNA expression. The aim of the study was to find interrelation between ACE and BDKRB2 gene polymorphisms and physical performance of elite Russian kayakers. Genotyping was performed by RT-PCR. Physiological parameters were evaluated by Kayak Ergometer and MetaLyzer II Gas Analyzer at the beginning and at the end of preparation period. Maximal oxygen consumption was increased by 12.7% and 14.8% in males and females, respectively. Furthermore, the ventilation volume (VE) was decreased by 9.6% in males. The total number of ACE I and BDKRB2 -9 alleles, favorable for endurance performance, was negatively correlated with VE values in males ( $p=0.0074$ ) and females ( $p=0.017$ ), indicating that these alleles are associated with the improvement of work economization of respiratory muscles (one of the indicators of aerobic capacity).

## P09.067

**European Lactase Persistence Allele is Associated With Increase in Body Mass Index**J. A. Kettunen<sup>1,2</sup>, K. Silander<sup>2,3</sup>, O. Saarela<sup>3</sup>, V. Anttila<sup>1</sup>, J. Laitinen<sup>4</sup>, A. Hartikainen<sup>5</sup>, A. Pouta<sup>5,6</sup>, P. Laheromo<sup>2</sup>, S. Männistö<sup>3</sup>, A. Jula<sup>7</sup>, J. Virtamo<sup>3</sup>, V. Salomaa<sup>3</sup>, G. Davey Smith<sup>8</sup>, M. I. McCarthy<sup>9,10</sup>, M. Järvelin<sup>11,12</sup>, M. Perola<sup>13,3</sup>, L. Peltonen<sup>1,2</sup>;<sup>1</sup>Wellcome Trust Sanger Institute, Cambridge, United Kingdom, <sup>2</sup>FIMM, Institute

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The global prevalence of obesity, usually indexed by body mass index (BMI) cut-offs, has increased significantly in the recent decades, mainly due to positive energy balance. However, the impact of a selection for specific genes cannot be excluded. Here we have tested the association between BMI and one of the best known genetic variants showing strong selective pressure: the functional variant in the cis-regulatory element of the lactase gene. We tested this variant since it is presumed to provide nutritional advantage in specific physical and cultural environments. We found that the variant responsible for lactase persistence among Europeans was also associated with higher BMI in a Nordic population sample ( $p = 1.3 \times 10^{-5}$ ) of 15 209 individuals, the size of the effect being close to that of FTO. We tested the effect of population stratification and concluded that the association was not due to population substructure.

## P09.068

**Association between copy number variation of glycogen synthase kinase 3 beta / Nr1i2 and major depression**Z. Elek<sup>1</sup>, E. Szantai<sup>1</sup>, R. Nagy<sup>1</sup>, G. Faludi<sup>2</sup>, A. Sarosi<sup>2</sup>, M. Sasvari-Szekely<sup>1</sup>;<sup>1</sup>Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest, Hungary, <sup>2</sup>Department of Clinical and Theoretical Mental Health, Kütvölgyi Clinical Center, Semmelweis University, Budapest, Hungary.

Copy number variation (CNV) or copy number polymorphism (CNP) is a novel approach in candidate gene studies. Recently, glycogen synthase kinase 3 beta (Gsk3β) and its adjacent gene, Nr1i2 (pregnane X receptor isoform) has been reported to associate with bipolar depression (Lachman et al, 2007). Here we present a case - control study of 216 patients with major depression and 175 controls, involving the chromosomal region of glycogen synthase kinase 3 beta (Gsk3β) and its adjacent genes, Nr1i2 and C3ORF15. The gene dosage has been measured by Taqman (Applied Biosystems) real time PCR systems, as well as by conventional PCR and capillary electrophoresis. In accordance with the previously published results, the variations in the copy number of the above genes seem to be very rare, although an accumulation of increased copy number has been found in the patient group (4/216 vs. 1/175). On the other hand we did not find any deletion of these genes in our samples. Taking together the published results of Lachman et al. and ours, amplification of this region seems to have a significant ( $p=0.006$ ) increase among patients with major depression.

## P09.069

**Significant associations between AKT1 SNP markers and Major Depressive Disorder in the Chinese population**Z. Z. Zhao<sup>1</sup>, X. Q. Chen<sup>2</sup>, D. Q. Li<sup>1</sup>, M. J. Wang<sup>1</sup>, M. Ai<sup>1</sup>, N. Chen<sup>2</sup>, J. M. Chen<sup>1</sup>, X. M. Li<sup>1</sup>, L. Kuang<sup>1</sup>;<sup>1</sup>The First Affiliated Hospital, Chongqing Medical University, Chongqing, China,<sup>2</sup>West China Hospital, West China Medical School, Sichuan University, Chengdu, China.

Background: V-akt murine thymoma viral oncogene homologue 1 (AKT1) is a serine/threonine kinase. Abnormality of AKT1 is involved in various diseases, including mental disorder. Recent evidence suggests that the Variation in AKT1 gene has been associated with schizophrenia, Parkinson's disease and type II diabetes. But the relationship of AKT1 gene variation in depression is unknown. The aim of the present study was to investigate the potential role of variability within AKT1 gene polymorphisms as a risk factor for major depressive disorder (MDD).

Method: We performed a case-control association analysis of AKT1. Five single nucleotide polymorphisms (SNPs) according to the origi-

nal study were genotyped among 127 MDD (DSM-IV criteria) and 127 healthy controls from the Chinese population. Samples were investigated by using PCR-RFLP.

Results: There were a positive association of allele T of the marker SNP3 with MDD (rs3730358,  $p = .024$ ). Haplotype analysis showed that the frequency of a four-AKT1 SNP1/2/3/4 haplotype (AGTG) was significantly higher in MDD patients (0.054) than that of controls (0.011) ( $p = .011$ ).

Conclusion: Our study provides support for the hypothesis that AKT1 is a susceptibility gene for MDD.

#### P09.070

##### Genotyping of the serotonin transporter promoter polymorphism in a Dutch major depression cohort

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Major Depressive Disorder (MDD) is a psychiatric disorder with high public health significance: it is currently the leading cause of disease burden in western civilization and the fourth cause on a global scale. People suffering from MDD show persistent dysphoria and additional cognitive symptoms.

Various theories about the causes of MDD exist and a genetic component is recognized in most of them. However, in complex disorders it is a challenge to discover high impact mutations and polymorphisms.

A genetic region that has been investigated in relation to neuropsychiatric disorders is serotonin-transporter-linked polymorphic region (5-HTTLPR). Serotonin itself has been implicated in the cause of MDD and thus regulation of serotonin availability by the serotonin transporter provides a region of interest. The 5-HTTLPR has a long variant and a short variant, where a deletion has taken place. Within the deleted region a single nucleotide polymorphism (SNP) can be found, rs25531. This SNP has also been investigated in relation to psychiatric illnesses, including MDD. Many of these studies are limited by a small sample size and might therefore not find verifiable associations.

In this study we use the MDD-cohort (3840 samples) of the genetic association information network (GAIN) to unearth possible associations between MDD and the 5-HTTLPR.

The region consists of repeats and has a high GC-content. For determining a long or short genotype we use fluorescent primers to amplify the region and separate products with capillary electrophoresis. Mspl was used to distinguish different genotypes of rs25531.

#### P09.071

##### Relationships between genes of interleukin 10 gene cluster and mood and anxiety disorders

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Major depressive disorder (MDD) and panic disorder (PD) belong to the most prevalent mental diseases, affecting respectively 10% and 3% of general population. Alterations in immune system have been implicated in the onset and development of MDD and PD. We studied the relationship between single-nucleotide polymorphisms (SNPs) of genes from interleukin 10 (IL10) chromosomal region 1q32 and MDD and PD. The association study design was used: 38 SNPs from 10 genes and regions between them of IL10 gene cluster were analyzed in 522 unrelated patients and in 356 healthy control subjects. All subjects were individuals of Caucasian origin living in Estonia. Patients were divided into two groups according to diagnosis: comparison of allelic frequencies was performed between control group and MDD patients (n=313), PD patients (n=209), and the whole patient group (n=522). Both MDD and PD groups included 'pure' phenotypes as well as phenotypes comorbid to other mood and anxiety disorders. SNplex Genotyping System was applied for genotyping, following association and haplotype analyses with Haplovview program. Association analysis of 38 SNPs revealed the most prominent relationship between MDD, PD, and the whole patient group and SNP (rs1539243) in IKBKE gene (inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase epsilon) (allelic p values 0.02, 0.0009, and 0.0011, respectively). Haplotype analysis revealed seven haplotype blocks in all tested groups of patients compared to healthy controls. None of frequencies of haplotypes differed significantly. Results show that IKBKE gene from 1q32 chromosomal region may possibly be related to mood and anxiety disorders.

Chromosome aberrations have long been studied in an effort to identify susceptibility genes for schizophrenia. Chromosome 22q11.2 microdeletion is associated with DiGeorge and Velocardiofacial syndromes (DG/VCF) and provides the most convincing evidence of an association between molecular cytogenetic abnormality and schizophrenia. In addition, this region is one of the best replicated linkage findings for schizophrenia. Recently, the reciprocal microduplication on 22q11.2 has been reported as a new syndrome. Preliminary data indicates that individuals with these duplications also suffer from neuropsychiatric disorders. In this study we have investigated the appropriateness of testing schizophrenia patients for the 22q11.2 microduplication. We used multiplex ligation-dependent probe amplification (MLPA) to measure copy number changes on the 22q11.2 region in a sample of 120 patients with schizophrenia. Our results corroborate the prevalence of the 22q11.2 microdeletion in patients with schizophrenia and clinical features of DG/ VCFs and do not suggest an association between 22q11.2 microduplication and schizophrenia.

#### P09.072

##### Failure to detect microduplication 22q11.2 among a group of Schizophrenia patients with Multiple Ligation Dependent Probe Amplification (MLPA)

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Chromosomal aberrations have long been studied in an effort to identify susceptibility genes for schizophrenia. Chromosome 22q11.2 microdeletion is associated with DiGeorge and Velocardiofacial syndromes (DG/VCF) and provides the most convincing evidence of an association between molecular cytogenetic abnormality and schizophrenia. In addition, this region is one of the best replicated linkage findings for schizophrenia. Recently, the reciprocal microduplication on 22q11.2 has been reported as a new syndrome. Preliminary data indicates that individuals with these duplications also suffer from neuropsychiatric disorders. In this study we have investigated the appropriateness of testing schizophrenia patients for the 22q11.2 microduplication. We used multiplex ligation-dependent probe amplification (MLPA) to measure copy number changes on the 22q11.2 region in a sample of 120 patients with schizophrenia. Our results corroborate the prevalence of the 22q11.2 microdeletion in patients with schizophrenia and clinical features of DG/ VCFs and do not suggest an association between 22q11.2 microduplication and schizophrenia.

#### P09.073

##### Role of the Estrogen Receptor (ESR1 Pvull and ESR1 325 C>G) and Progesterone Receptor (PROGINS) polymorphisms in genetic susceptibility to Migraine in a North Indian Population

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We aimed to explore the single-locus, haplotype, epistasis patterns and contribution of ESR1 Pvull (rs2234693), ESR1 325 C>G (rs1801132) and PROGINS (rs1042838) polymorphisms in genetic susceptibility to migraine by analyzing 613 subjects consisting of 217 Migraine patients, 217 Healthy controls (HC) and 179 patients with Tension Type Headache (TTH). Entire data was analyzed by taking Bonferroni corrected P value into account. We found significant association of TT genotype (OR=3.458; CI=1.757-6.806; P value=0.0003) and T allele (OR=1.729; CI=1.309-2.284; P value=0.0001) of ESR1 Pvull SNP with migraine when compared with HC. Significant association was seen only in female migraine patients. Moreover, higher risk of TT genotype and T allele was limited to migraine with aura (MA) than migraine without aura (MO). In case of ESR1 Pvull, risk in migraine patients could fit in the recessive model, but no risk was observed when TTH patients were compared with HC. However, in ESR 325 G>C polymorphism, no significance was seen in any of the models. In PROGINS polymorphism, significant low risk at genotypic and allelic levels was seen when migraine patients were compared with HC. ESR1 Pvull TT \* ESR1 325 C>G CG genotype, PROGINS A1A2 \* ESR1 325 C>G CG genotype and ESR1 Pvull CT \* PROGINS A1A2 interacted significantly, but the significance was lost after Bonferroni correction. In conclusion, ESR1 Pvull polymorphism is a significant risk factor for migraine particularly in females and MA patients, but, ESR 325 C>G and PROGINS polymorphisms are not associated with migraine susceptibility in North Indian population.

#### P09.074

##### Association of a non-coding mtDNA polymorphism with longevity in Russian population

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Due to key role of mitochondria in cell energy production, mitochondrial DNA is considered as candidate locus for different common diseases as well as for predisposing to longevity. Some associations of different mtDNA haplogroups with longevity were shown in European populations. To explore possible associations of mtDNA polymorphisms with longevity in population of Siberian part of Russia, we have studied mtDNA in samples of long-livers (>90 years old) from Tomsk and Novosibirsk (N=235) by sequencing HVS1 region and restriction analysis for haplogroup assignment. We have compared frequencies of main European haplogroups (H, H1, U4, U5, K, J, T) and some frequent polyphyletic HVS1 polymorphisms (16189, 16311, 16362, etc) in long-livers group with mtDNA data on Tomsk population. No significant differences were found for haplogroups, as well as for big clusters (J+T, U, H+HV+V). At the same time, the long-livers had higher frequency of T16189C polymorphism (21.3% comparing to 12.2% in population, P=0.04). There was no difference between long-livers and population in "haplogroup composition" of the 16189C group (main contributors were haplogroups H1 and U5). T16189C variant is known as associated with diabetes in adults, increased body mass index and insulin resistance. In contrast, our results suggest that this variant may have also positive influence on fitness, thus favoring for longevity. The study was supported by RFBR grant 07-04-01526a.

#### P09.075

#### Infant C677T genotype of the MTHFR gene as a risk factor non-syndromic cleft lip with/ without palate

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5,10- Methylentetrahydrofolate reductase (MTHFR) gene is located on 1p36.3 and involved in folate metabolism. It has two common gene variants, C677T and A1298C. C677T homozygosity is associated with several congenital anomalies, mostly neural tube defects. Non-syndromic cleft lip with/without palate (NSCLP) is one of the most common congenital anomalies, with a prevalence of 1/1000 in Caucasians. It is a multifactorial pathology affected from different loci and genes, environmental factors also implicated in NSCLP. To date C677T, in the terms of NSCLP and MTHFR, has either conflicting results or couldn't be replicated properly. Our aim is to determine a relation between infants' MTHFR C677T polymorphism and (NSCLP) in Turkish population.

We have established 100 NSCLP patients without any family history of CLP and 100 controls. PCR- RFLP method was performed for the determination of MTHFR gene by using specific primers and Hinf I restriction enzyme.

CC (wild type) frequencies were %39 and %41 in NSCLP patients and controls respectively. %43 of the patients and %47 of the controls were CT and %18 of the patients and %12 of the controls were TT (mutant) for the MTHFR C677T allele.

No statistically significant differences were detected between study and control groups. Our study suggests that only MTHFR C677T genotyping in infants may be a useless approach to suggest as a risk factor for NSCLP in Turkish population.

#### P09.076

#### The MTHFR C677T polymorphism increase the risk for renal involvement in type 2 diabetic patients

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Renal disease is one of the most common complications of diabetic patients. Many studies suggest that MTHFR (Methylentetrahydrofolate reductase) and TGF-beta polymorphisms increases the risk for renal involvement in diabetic patients.

The purpose of this case-control study was to estimate the association

between MTHFR C677T and TGF-beta C-509T polymorphisms and renal failure in diabetic patients.

Clinical information and biological samples were collected from dialyzed patients with type I diabetes (n=116, male: 56%, dialysis: 1,6±0,8 years), type II diabetes (n=123, male: 55.28%, 2,4±1,2, dialysis: 2,3±1,2 years) or chronic glomerulonephritis (n=121, male: 49.58%, dialysis: 1,7±0,9 years) and from healthy subjects (n=494, fasting glycemia 93.2±8.2 mg/dl). Healthy subjects were selected to be matched for age and gender with patients. All subjects selected for this study were unrelated Romanian Caucasians.

Blood samples from all subjects were used for DNA extraction. DNA samples were used for genotyping TGFb -509 and MTHFR C677T polymorphisms using PCR and PCR RFLP methods.

In the case of MTHFR C677T the distribution of genotypes remains significant only when T2DM patients and controls were compared (p=0,019). The results showed no association between TGFbeta and the risk for renal failure in diabetic or nondiabetic patients (OR~1).

In conclusion, our study demonstrates:

1. The MTHFR C677T is associated with ESRD in type 2 diabetic patients.
2. TGF-beta genotypes are not significant risk factors for development of ESRD.

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#### P09.077

#### An investigation of polymorphisms in CTLA-4 gene for association with multiple sclerosis in Iranians

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Multiple sclerosis (MS), a chronic inflammatory demyelinating disease of the central nervous system is believed to have a T cell-mediated autoimmune etiology. The cytotoxic T lymphocyte antigen 4 (CTLA-4) gene is a strong candidate for the involvement in autoimmune diseases. To examine the genetic association of the CTLA-4 gene locus with MS, in a case-control design, we analyzed three single nucleotide polymorphisms (SNPs) of the CTLA-4 gene including -318 C/T, +49A/G and CT60 in 135 unrelated Iranian relapsing-remitting MS patients and 135 healthy subjects using PCR-RFLP method. The overall genotype frequencies of -318 CC, CT and TT were 79.6%, 18.9% and 1.5%, respectively. Regarding the +49A/G SNP the overall genotype frequencies for AA, AG and GG were 29.6%, 53% and 17.4%, respectively and for CT60 SNP the overall genotype frequencies for AA, AG and GG were 19.3%, 50.7 and 30%, respectively. The distribution of CTLA-4 (-318 C/T) genotype and allele frequencies did not significantly differ between MS patients and healthy subjects. In conclusion, there may not be any association between CTLA-4 gene polymorphisms and MS development.

Key words: Allelic association; CTLA-4; multiple sclerosis; RFLP; Single nucleotide polymorphism

#### P09.078

#### Polymorphisms of hemochromatosis and transferrin genes in multiple sclerosis

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Recent evidence has indicated a role for iron dysregulation in disease pathogenesis. We tested the hypothesis that polymorphisms in HFE (C282Y and H63D) and TF (C1 and C2) genes, and interactions among these polymorphisms, influence predisposition to and clinical presentation of multiple sclerosis (MS).

Three hundred and sixty-eight MS patients and 368 healthy controls were genotyped by PCR-RFLP method.

Statistically significant higher frequency of the C282Y mutation carriers was observed in patients with secondary-progressive or relapsing-remitting MS (7.7%) than in the control group (3.8%) (p=0.026). A significantly earlier age of onset was found in carriers of the C282Y mutation (p=0.035). The frequency of H63D homozygotes was higher (2.8%) in control group than in MS patients (0.7%) with borderline significance (p=0.050). We found no correlation between H63D mutation and disease behavior (p>0.05). We were unable to detect significant

differences ( $p>0.05$ ) in the frequencies of TF C1/C2 genotypes and alleles between MS patients and controls, and we found no differences in clinical parameters in carriers of the C2 allele.

Our results indicate that C282Y mutation may be risk factor with respect to MS susceptibility and probably a good predictor for early onset of MS. The possible protective effects of H63D polymorphisms was less pronounced. Regarding MS susceptibility or disease progression no gene-gene interaction between HFE and TF could be established.

#### P09.079

##### Association study of the Interleukin-2/Interleukin-2 receptor alpha (IL2/IL2RA) system with multiple sclerosis

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IL2/IL2RA system is involved in T helper and T suppressor cell activity and has a major role in the immune response and in the control of autoimmunity. It has been reported association of the IL2 and IL2RA loci with several diseases as type 1 diabetes (T1D), celiac disease and multiple sclerosis (MS) in independent studies. This overlap of risk loci among autoimmune diseases raises the possibility of sharing common or distinct pathological mechanism that has to be deciphered. We performed a case-control association study in MS with 805 patients and 952 matched Caucasian controls from the South of Spain with several polymorphisms in these loci. We have found differences between the polymorphisms associated and risk alleles among all of them. Our results replicate and extend the association found in the IL2/IL2RA loci with MS. These differences with other diseases may reflect distinct roles that such gene variants may have in these pathologies.

#### P09.080

##### Interferon regulatory factor 5 (IRF5) gene variants are associated with multiple sclerosis in three distinct populations

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Multiple sclerosis (MS) is an inflammatory disorder that mainly damages the central nervous system. Here we investigated whether this disease would be associated with the variants of interferon regulatory factor 5 (IRF5) gene, which encodes a transcription factor involving both in the type I interferon and the toll-like receptor signaling pathways. In total nine single nucleotide polymorphisms (SNPs) and one insertion-deletion polymorphism (indel) in the IRF5 gene were genotyped in a collection of 2337 patients with MS and 2813 controls from three populations: two case-control cohorts from Spain and Sweden, and a set of MS trio families from Finland. Two SNPs (rs4728142, rs3807306), as well as the 5 bp indel located in the promoter and first intron of the IRF5 gene, showed association signals with values of  $p<0.001$  when the data from all cohorts were combined. The predisposing alleles were present on the same common haplotype in all populations. Using electrophoretic mobility shift assays we observed allele specific differences in protein binding for the SNP rs4728142 and the 5 bp indel, and by a proximity ligation assay we demonstrated increased binding of the transcription factor SP1 to the risk allele of the 5 bp indel. These findings add IRF5 to the list of genes shown to be associated with MS. As IRF5 has already been revealed to be related to several diseases with features of autoimmunity, our study adds to the evidence the type I interferon system is likely to be involved in the development of these diseases.

#### P09.081

##### Common single nucleotide polymorphisms in the Interleukin-7receptor $\alpha$ gene and their associations with variable expression of IL7R $\alpha$ in Iranian population of multiple sclerosis patients

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Multiple sclerosis (MS) is an enigmatic disease of the central nervous system resulting in sclerotic plaques with the pathological hallmarks of demyelination and axonal damage. Susceptibility to MS involves a genetically complex autoimmune component. However, except for

genes in the HLA system, specific susceptibility loci were unknown or unconfirmed yet. In recent studies in some countries it has shown that allelic association of polymorphisms in the gene encoding Interleukin 7 receptor  $\alpha$  chain (IL7Ra) is a significant risk factor in MS. In this study we investigated the expression and possible related haplotypes of five SNPs in IL7Ra gene with MS in a population from Iran.

In this study we performed expression analysis of IL7Ra gene by semi quantitative RT-PCR in MS patients and controls using special designed primers to distinguish the expression level of soluble and trans-membrane isoforms. In addition we did genotyping of three SNPs in promoter region and two SNPs in inside of the gene to figure out the possible haplotype patterns. Our result shows that the IL7Ra expression of soluble isoform is higher than trans-membrane isoform in MS patients in compare to control group. Our data also indicated that some special haplotypes are more frequent in MS patients than to controls.

#### P09.082

##### TNF- $\alpha$ promoter polymorphisms in multiple sclerosis: no association with -308 and 238 alleles, but the -857 alleles in associated with the disease in Turkish patients

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Dysregulation in the expression of pro-and anti-inflammatory cytokines is one of the milestones in multiple sclerosis (MS) development and progression. Tumor necrosis factor (TNF- $\alpha$ ), a proinflammatory cytokine is believed to play an important role in MS pathogenesis. The objective of this study is to investigate the association between TNF- $\alpha$  promoter region (TNF- $\alpha$  -238, -308 and -857) and susceptibility to MS and clinical course of the disease. Eighty six relapsing remitting MS patients and 150 sex, age and ethnic matched controls were enrolled in the study. Genotyping was performed by PCR-RFLP method.

We observed a statistically significant increase in TNF- $\alpha$  857 CC genotype in MS patients than controls ( $p<0.001$ ) while TNF- $\alpha$  857 CT genotype showed a significant negative correlation with MS patients ( $p=0.033$ ). No differences in the distribution of the TNF- $\alpha$  238 and 308 alleles were observed. None of the three polymorphisms (-238,-308,-857) did not show relation with disease duration, EDSS or age of onset. On the other hand significant difference of TNF-857 CC genotype was identified with the low disease index ( $p=0.025$ ).

Although the study group is small, the results indicate that TNF- $\alpha$  857 CC genotype may cause susceptibility to MS in the Turkish population.

#### P09.083

##### Musical aptitude and creativity in music are associated with AVPR1A-haplotypes

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Artistic creativity forms the basis of music culture and music industry. Composing, improvising and arranging music are complex creative functions of the human brain, which biological value remains unknown. We hypothesized that practicing music is social communication that needs musical aptitude and even creativity in music. We analyzed polymorphisms of the arginine vasopressin receptor 1A (AVPR1A), serotonin transporter (SLC6A4), catecol-O-methyltransferase (COMT), dopamin receptor D2 (DRD2) and tyrosine hydroxylase 1 (TPH1), genes associated with social bonding and cognitive functions in 19 Finnish families (n=343 members) with professional musicians and/or active amateurs. All family members were tested for musical aptitude using the auditory structuring ability test (Karma Music test; KMT) and Carl Seashores tests for pitch (SP) and for time (ST). Data on creativity in music (composing, improvising and/or arranging music) was surveyed using a web-based questionnaire.

We show that creative functions in music have a strong genetic component ( $h^2 = .84$ ; composing  $h^2 = .40$ ; arranging  $h^2 = .46$ ; improvising  $h^2 = .46$ ) and that high music test scores are associated with creative functions in music ( $p<.0001$ ). We discovered a significant haplotype association with AVPR1A and KMT ( $p=0.0008$ ), and combined music scores (COMB) ( $p=0.0056$ ), ST ( $p= 0.0038$ ) and COMB ( $p=0.0083$ )

using HBAT. AVPR1A alleles and haplotypes were also associated with composing ( $p=0.0351$ ), improvising ( $p=0.0364$ ) and arranging music ( $p=0.0334$ ). Serotonin transporter gene (SLC6A4: promoter region 5-HTTLPR) was highly associated with COMB ( $p=0.0084$ ). The results suggest that the neurobiology of music perception and production is related to the pathways affecting intrinsic attachment behavior important in evolution.

#### P09.084

##### No association of oestrogen receptor gene polymorphisms with myasthenia gravis

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Objective: Myasthenia Gravis (MG) is an autoimmune disease that affects women more often than men, implying that sex hormones may play a role in its pathogenesis. Our aim was to investigate the possible role of oestrogen in the pathomechanism of MG by evaluating the association of oestrogen receptor (OR) polymorphisms in MG.

Methods: Pvull and XbaI restriction fragment polymorphisms of the OR gene were analyzed in 33 male and 130 female MG patients and 40 male and 134 female healthy controls. The distribution of OR genotypes was compared between the MG patients and the healthy control group. MG patients were divided into groups according to their OR genotypes, then their AchR antibody status and age of onset and were compared between the groups.

Results: The OR genotype frequency of distribution did not show any significant differences either between male MG patients and male controls or female patients and female controls. No association was found between the OR status and the age of onset or AchR antibody positivity in either of the genders.

Conclusion: Though a larger patient sample is needed, our results suggest, that OR status does not influence MG age of onset or AchR antibody seropositivity.

#### P09.085

##### Association of the SPINK5 gene genotypes and haplotypes with nasal polyposis

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Nasal polyps (NP) are lesions that emanate from nasal mucosa or paranasal sinuses, caused by inflammation, related to dysregulation of epithelial cell proliferation. NP incidence is higher in cystic fibrosis (CF) patients than in general population (35-45% vs 1-4%) and is one of the major otolaryngological CF manifestations, but no particular CFTR gene mutation was found associated with NP in CF patients. In our previous study the D386N-SNP in SPINK5 gene was found associated with NP in CF patients. The aim of this study was to determine if SPINK5 variants could modulate the development of NP in CF patients. Five SNPs, representative of SPINK5 LD blocks, were selected and analysed in 4 groups of subjects: 79 nonCF subjects with NP and 39 CF patients with NP, 104 CF patients without NP and 101 controls. All CF patients have two CFTR severe mutations. Allele and genotype frequency and HW equilibrium were calculated for each polymorphism. An association analysis between either single SNP or 5 SNPs of the SPINK5 gene and the NP was carried out. Haplotype analyses were also computed.

A significant association between rs6892205 and NP was found in almost all the analyses performed ( $p: 0.005-0.01$ ) while association of D386N was limited to CF group only ( $p=0.001$ ). A preferential association with NP was also confirmed by the haplotype analysis ( $p: 0.0015-0.015$ ). These data support the association of SPINK5 gene with NP. It is possible to suppose for SPINK5 gene a role in the inflammatory process involved in the pathogenesis of NP.

#### P09.086

##### Maternal MTHFR polymorphisms do not predispose to neural tube defects in India

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MTHFR polymorphisms C677T and A1298C are reported to predispose to neural tube defects (NTDs) in the western world. We studied the association of these SNPs in 175 Indian women who carried fetuses affected with NTDs and 332 unaffected fetuses (control). We also measured maternal plasma folate, vitamin B12 and homocysteine concentrations. Folate deficiency was observed only in 6% of mothers who carried NTD fetuses. There was no association of maternal MTHFR SNPs with plasma folate, vitamin B12 or homocysteine concentrations in cases, while in controls; TT genotype at C677T was associated with significantly higher homocysteine concentrations.

Frequency of the risk allele T at the C677T polymorphism was 0.13 in mothers with NTD fetuses and 0.14 in controls, while that of the risk allele C at the A1298C polymorphism was 0.36 in mothers with NTD fetuses and 0.47 in controls.

The 677T allele in mothers was not associated with NTDs in the offspring ( $p=0.68$ ) while the 1298C allele was more frequent in mothers who had unaffected offspring ( $p=0.002$ ). 1298C allele was noted to be preferentially transmitted from mother to offspring in 75% of cases and 80% of controls, while 677T allele was preferentially transmitted from mother to offspring in 60% of cases and 56% of controls. There was no significant association between the above MTHFR polymorphisms and NTDs in the offspring.

Our results suggest that the MTHFR polymorphisms which predispose to NTDs in the western populations have limited contribution to the etiology of NTDs in India.

#### P09.087

##### The common MTHFR C677T and A1298C variants are not associated with the risk of non-syndromic cleft lip/palate in northern Venezuela

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Non-syndromic cleft lip with/without palate (nsCL/P) is among the most common of major birth defects, with a frequency of 1/500-1/2000 in most populations and with complex inheritance probably involving multiple genes and environmental factors. Numerous studies of MTHFR, encoding methylenetetrahydrofolate reductase, which catalyzes the rate-limiting step of folic acid biosynthesis, have yielded inconsistent association of two common hypomorphic allelic variants, C677T and A1298C. Some showed apparent allelic association between MTHFR polymorphisms and nsCL/P in patients versus controls, and others finding no or variable association. Other studies have reported association in mothers of patients with nsCL/P. Also, contribution of mothers genotype to the risk of nsCL/P has been reported. We have studied the two common functionally significant MTHFR variants, C677T and A1298C, in nsCL/P patients ( $n=179$ ), their mothers ( $n=168$ ), and population-matched controls ( $n=138$ ) from northern Venezuela. We found no supporting evidence for contribution of both the MTHFR C677T and A1298C polymorphisms to the risk of non-syndromic cleft lip with/without palate in the population studied. Altogether, our data do not support a causal role of either the MTHFR C677T or A1298C polymorphisms in the pathogenesis of non-syndromic cleft lip with/without palate risk in northern Venezuela.

#### P09.088

##### Influence of main, interaction and modified effects of NET, ADRA2A and COMT gene polymorphisms on personality traits in healthy individuals

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Since psychobiological model of personality was proposed by Cloninger, interest of scientific groups has been focused on neurotransmitter system genes. We aimed to define a single genotype effect of NET 1287A/G, ADRA2A -1291C/G and COMT Val158Met polymorphisms and to check possible epistatic effect between them and personality traits (assessed with the EPI and TCI questionnaires).

We recruited 652 healthy individuals (men-222, women-430) of Caucasian origin (Russians-233, Tatars-419) from Russia (mean age:  $19.53 \pm 2.24$  years). Genotyping of 3 polymorphisms was performed using PCR-RFLP. The main, interaction and modified effects of gene polymorphisms, gender and ethnicity on personality traits were detected under multiple regression (MR) analyses (SPSS 13.0).

While conducting MR analyses, the main effect of ethnicity ( $P < 0.0001$ ) on variations in Extraversion, ADRA2A -1291C/G ( $P = 0.028$ ) and ethnicity ( $P < 0.0001$ ) on Neuroticism, gender ( $P < 0.0001$ ) and ethnicity ( $P = 0.002$ ) on Novelty Seeking, NET 1287A/G ( $P = 0.007$ ) and gender ( $P = 0.037$ ) on Harm Avoidance, gender ( $P < 0.0001$ ) on Reward Dependence, NET 1287A/G ( $P = 0.047$ ) on Persistence was observed. Moreover, epistatic effect of gene-gene and gene-environment interaction was established: ADRA2A\*NET\*COMT\*ethnicity\*gender interaction ( $P = 0.026$ ) and ADRA2A\*gender\*ethnicity modified effect ( $P < 0.0001$ ) explained 0.9% and 8% of variance in Neuroticism correspondingly; ADRA2A\*COMT interaction ( $P = 0.024$ ) explained 1.3% of variance in Novelty Seeking; ADRA2A\*COMT interaction ( $P = 0.005$ ) and NET\*gender\*ethnicity modified effect ( $P = 0.001$ ) corresponded to 1.7% and 2.4% of variance in Harm Avoidance accordingly; COMT\*ethnicity interaction ( $P = 0.004$ ) explained 1.4% of variance in Reward Dependence.

Our findings indicate that noradrenergic system genes have larger impact on anxiety-related traits than on Reward Dependence. Study was supported by Russian foundation for humanities grant (08-06-00579a).

#### P09.089

##### Genotypes-by-nutrient associations of common polymorphisms in obesity-related genes within the Czech population

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**INTRODUCTION:** The personal food preferences can either enhance or suppress the development of obesity and the selection and proportion of macronutrients in the diet seems to have a heritable component. In this study, we therefore focused on dietary composition as a specific trait related to obesity and we determined, whether genetic variations in leptin (LEP), leptin receptor (LEPR), ghrelin (GHL), adiponectin (ADIPOQ), interleukin 6 (IL6), proopiomelanocortin (POMC) and melanocortin-4 receptor (MC4R) underlie specific native food preferences and obesity-related anthropometric parameters.

**METHODS:** The total of 409 individuals of Czech Caucasian origin were enrolled into this study and the 7-day food records and parallel 7-day records of physical activity were obtained from the study subject as well as anthropometric measurements. In a subset of study subjects, plasma levels of adiponectin, leptin and soluble leptin receptor were measured.

**RESULTS:** Independently on the BMI of the individuals, common variations in leptin and leptin receptor genes were associated with specific eating patterns, mainly with respect to timing of eating. Diastolic blood pressure was significantly associated with the common variation in the leptin receptor gene as well as with the ratio of soluble leptin receptor and leptin plasma levels. In multivariate analysis, common variations in LEP, LEPR, POMC and MC4R genes expressed an independent prediction role for percentage of body fat.

**DISCUSSION:** To conclude, we report common allelic variants associated with specific feeding behaviour and obesity-related anthropometric traits. Moreover, we identified allelic variants that significantly influence time structure of food intake during the day.

#### P09.090

##### Genes and lifestyle factors in obesity: results from 14,000 subjects from KORA

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**Background:** Data from meta-analyses of genome-wide association studies provided evidence that genetic variants are associated with body mass index (BMI). For most of these genes, expression results indicated a neural role. The aim of our study was to investigate these loci in a homogenous population-based study not only for their association with BMI and percentage body fat, but also with lifestyle factors. **Methods:** 7,080 women and 6,977 men from the population-based KORA study were genotyped for polymorphisms in or near the genes being associated with BMI (e.g. *FTO*, *MC4R*, *TMEM18*, *SH2B1*). Nutritional parameters (carbohydrate and fat intake scores), physical activity scores, smoking behaviour, and alcohol consumption were derived from detailed questionnaires. For statistical analysis, regression-based models were used.

**Results:** The minor allele of polymorphism rs1558902 (T>A) in the *FTO* gene was associated with higher BMI ( $p = 1.37 \times 10^{-7}$ ) and percentage body fat ( $p = 0.0002$ ). Also polymorphism rs6548238 within *TMEM18* gene was associated with BMI (T allele  $-0.42 \text{ kg/m}^2$ ,  $p = 1.22 \times 10^{-8}$ ) and percentage body fat (T allele  $-0.41\%$ ,  $p = 0.0002$ ). For lifestyle factors, no significant association with any of the analysed DNA variants was observed after adjustment for multiple testing. Accordingly, the association between genotypes and BMI was not influenced by taking lifestyle factors as a covariate into the model. Similar results were found for other genes and polymorphisms.

**Conclusion:** In our homogenous large population-based study previously detected obesity associated genes were significantly associated with BMI and percentage body fat. There is no evidence for an association of any of these polymorphisms with lifestyle factors.

#### P09.091

##### The Gln241His polymorphism in the carbohydrate response element binding protein (MLXIPL) gene is associated with fasting triglyceride concentrations and BMI in a Mediterranean population

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In a recent genome-wide association study, we participated in the identification for the first time of a new locus associated with plasma triglyceride concentrations. This loci was at 7q11 near *TBL2* (transducin (beta)-like 2) and *MLXIPL* (MLX interacting protein-like or carbohydrate response element binding protein) genes. Further studies identified a nonsynonymous SNP (rs3812316, G771C, Gln241His) in the *MLXIPL* gene as the SNP most significantly associated with plasma triglycerides. Moreover, its has been suggested that *MLXIPL* is a thrifty gene because the wild-type variant may permit more efficient food utilization, fat deposition and rapid weight gain at times of food abundance. Therefore our aims were to study the association between the Gln241His in the *MLXIPL* gene with fasting triglycerides and obesity-related variables in a high cardiovascular risk Mediterranean population.

We analyzed 1002 high cardiovascular risk individuals from the PRE-DIMED-Valencia Study. Participants (men and women aged 67y) were free of CVD and had type 2 diabetes (45%), or three or more CVD risk factors. Genetic, clinical, biochemical, anthropometric and life-style data were determined.

Prevalence of the *MLXIPL* genotypes were: 85.4% CC, 13.7% GC, 0.9% GG (allele frequencies, C=0.923 and G=0.077). Plasma triglyceride concentrations were significantly lower in carriers of the G allele ( $P = 0.012$ ). Interestingly, we found a consistent association of the SNP with lower BMI. In terms of weight, we observed a gene-dosage decreasing effect of this polymorphism: 78+/-13 kg (CC), 75+/-12 kg

(CG), 66+/-19 kg (GG); P=0.004. This decrease in body-weight in G-allele carriers was observed in both men and women.

#### P09.092

#### Serotonergic polymorphisms in childhood onset Obsessive Compulsive Disorder

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Childhood onset Obsessive Compulsive Disorder (OCD) has substantial genetic background. The symptoms are likely to evolve as a result of interactions of adverse environmental factors and predisposing genetic factors. Hitherto, only the long-allele of the serotonin transporter gene promoter polymorphism has been indicated as potential risk factor for OCD. In the present study we aimed to investigate genetic polymorphisms in the serotonin transporter, tryptophan hydroxylase 2 (TPH2), and serotonin 1A and 1B receptor genes.

Clinical diagnosis of 115 OCD patients was obtained by the DSM-IV criteria, for symptom assessment the Children's Yale-Brown Obsessive Compulsive Scale was administered. Anxiety or mood disorder was present in 38%, and 48% of the children had comorbid tics. Variable number of tandem repeats in the serotonin transporter gene (5HTTLPR in the promoter region, STin2 in the second intron) and single nucleotide polymorphisms in the TPH2 and serotonin receptor genes were studied. Allele- and genotype-frequencies of the OCD clinical group as well as subgroups of OCD with/without tics or anxiety disorder were compared to a sex matched (77.2% male and 22.8% female) control group.

The serotonin transporter polymorphisms were associated with OCD, more specifically with the OCD + anxiety disorder subgroup. Genotype frequency of the STin2 was significantly different when the anxiety present OCD group was compared to the anxiety absent OCD group or to the control group (p = 0.04, and p = 0.05, respectively). In addition, increased SL genotype frequency of 5HTTLPR could be detected in this subgroup.

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#### P09.093

#### AXIN2 contributes to cleft lip and palate in multiple populations

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Cancer and congenital malformations occasionally may have a common etiology. We have recently demonstrated that families segregating oral clefts present a higher incidence of family history of cancer and that AXIN2, a gene that when mutated increases susceptibility to colon cancer, was associated with cleft lip with or without cleft palate (CL/P) in a US population. In this study, we genotyped 484 families with more than two affected cleft individuals from **North America** (USA), **Europe** (Spain, Hungary and Turkey), **Asia** (China and India) and **Central America** (Guatemala) to test for association with AXIN2. TaqMan chemistry was used to generate genotype data in four SNP markers and alleles at each AXIN2 SNP were tested for transmission distortion with CL/P using the Family-Based Association Test (FBAT). We tested each population individually and pooled for all populations as well as for Caucasian, Asian, and Hispanic subsets. AXIN2 markers were associated with CL/P in the U.S. group (p=0.01), in the Spanish cohort (p=0.04), and in the pooled dataset (p=0.02). The association was more significant in the U.S. group when only cleft lip and palate individuals were considered in the analysis (p=0.003). Our results suggest that AXIN2 represents a component in the etiology of human clefting.

#### P09.094

#### Identification of genetic risk factors for preventing osteoporosis-related fractures

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Osteoporosis is a polygenic disorder dependent on multiple genes and environmental factors. It is one of the major and growing health care

problems around the world, related to the general aging of societies, with improvement in preventive health and delay in mortality. Fracture, the clinical outcome of osteoporosis, is also dependent on genetic factors, with a heritability of about 25-35%. The objective of the present study was to describe genetic epidemiological aspects of osteoporosis, as family history may predict the disease risk in otherwise healthy women. 62 patients were studied, mean age of 51 years, just before or soon after menopause. 2/3 of the women who had mothers with osteoporosis developed this disease too, which proves that the maternal parent with osteoporosis represents an important risk factor, unfortunately not possible to influence. About 1/3 of these women had vertebral osteoporosis, half of them having 1 or more vertebral fractures. Osteoporosis was developed in more than 2/3 of the cases in which menopause was settled before the age of 45. Thus, settling of menopause before this age is a very important cause for the release of physiopathological mechanisms of osteoporosis. A parental history of fracture confers an increased risk of fracture that is independent of bone mineral density, suggesting that there is another shared component, which influences bone fragility. These results provide an initial epidemiological profile for osteoporosis and fracture risk besides information useful for genetic counseling.

#### P09.095

#### Genetic associations of the antioxidative enzyme glutathione peroxidase 1 with low bone mineral density in men

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Glutathione peroxidase 1 (GPX1) is an important antioxidant enzyme in humans. It protects human body from negative effects of reactive oxygen species. Recently, oxidative stress has been suggested to participate in the development of osteoporosis. Osteoblasts can produce antioxidants such as GPX1 to protect against reactive oxygen species.

The aims of this study were to determine frequencies of genotypes of GPX1 genetic polymorphisms and to associate with low bone mineral density (BMD) and concentration of plasma osteocalcin of 115 men, aged  $67.96 \pm 6.01$  years. Each patient was examined clinically with the measurement of BMD and concentration of plasma osteocalcin.

PCR was used to amplify two fragments in coding regions of the gene GPX1 to determine polyalanine regions of 5, 6 and 7 tri-nucleotide repeats using the DHPLC method and Pro198Leu polymorphism using RFLP method.

The frequencies of genotypes of 115 men subjects were as follows: 5/5 (27.8%), 5/6 (19.1%), 5/7 (22.6%), 6/6 (9.6%), 6/7 (13.9%), 7/7 (7.0%) for the polyalanine repeats and CC (54.8%), CT (35.7%), TT (9.6%) for Pro198Leu polymorphism. The genotype 6/7 is significantly associated with a higher BMD value of femoral neck and total hip than the 5/5, 5/6, 5/7 and 6/6 genotypes ( $p < 0.04$ ). The borderline significant difference between CC and CT genotypes of Pro198Leu and BMD of total hip was detected ( $p = 0.052$ ). Moreover, no significant associations with plasma osteocalcin concentrations were determined.

The results show for the first time the significant association of the anti-oxidative genetic polymorphisms of GPX1 with the osteoporosis.

#### P09.096

#### Evidence of Epistasis Between the Catechol-O-Methyltransferase and Aldehyde Dehydrogenase 3B1 Genes in Paranoid Schizophrenia

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Background: Schizophrenia is a common yet severe psychiatric condition characterized by complex genetic mechanism and diverse clinical presentations. Our previous study indicated that the combined effect of two intronic single-nucleotide polymorphisms (SNPs), which are located in the catechol-O-methyltransferase (COMT) and aldehyde dehydrogenase 3B1 (ALDH3B1) genes, respectively, conferred genetic risk to paranoid schizophrenia.

Methods: To further explore the precise mechanism of the COMT and ALDH3B1 interaction involved in the pathophysiology of schizophre-

nia, we scanned all possible functional SNPs within these two genes by polymerase chain reaction (PCR)-based genotyping analysis in 540

paranoid schizophrenic patients and 660 control subjects from a Han Chinese population. We also determined the effects of schizophrenia associated SNPs on the development of psychotic symptoms, P300 event-related potential components induced by an auditory odd-ball task, and gene expression examined by quantitative real-time PCR analysis.

Results: The major findings of this study were that, among the individuals carrying the rs3751082 A allele in the ALDH3B1 gene, the rs4633 T allele in the COMT gene was associated with susceptibility to paranoid schizophrenia ( $p=0.004$ ), development of hallucination ( $p=5.141$  E-5), delay of P300 latency in both patients ( $p=0.006$ ) and control subjects ( $p=0.02$ ), and increased expression of the COMT gene in control subjects ( $p=0.002$ ). However, the rs4633 T allele did not show any association in the rs3751082 G/G genotype carriers.

Conclusions: These findings provided convincing evidence that epistasis between the COMT and ALDH3B1 genes plays an important role in the pathogenesis of schizophrenia.

#### P09.097

#### N-acetylation factor in the pathogenesis of pelvic organ prolapse (POP)

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More than half of women having children experience different form of prolapse in their late adulthood. A number of studies are focused on the POP pathogenesis. Metabolic enzymatic activities contribute greatly to the POP genesis. We have hypothesized that the activity of N-acetyltransferase-2 (NAT-2), a previously known factor for some gynecological pathology, could influence POP pathogenesis. Polymorphism of NAT-2 gene (NAT2\*4 (N), NAT2\*11A (S1), NAT2\*6B (S2), NAT2\*7A (S3)) was detected by PCR in 70 POP patients (age  $51.7 \pm 9.8$ ) and in population sample from Northwest region of Russia (89 individuals). Patients demonstrated different stages of POP: 31.4% - stage I, 34.3% - stage II, 28.6% - stage III and 5.7% - stage IV. In the patient group frequency of N/N genotype was significantly lower ( $p = 0.0015$ ) and frequency of homo- and heterozygous mutant alleles was significantly higher ( $p=0.0466$ ), compared to the population data. Interestingly, the frequency of S2 allele was significantly higher ( $p<0.0001$ ) in the patient group. Individuals from both patient and population group, having at least one N allele, were considered "rapid" acetylators and the rest as "slow" acetylators. According to our results, the rate of "slow" acetylators was higher among patients when compared to the population control. Thus, prevalence of mutated alleles among the POP patients supports our idea that the factor of "slow" N-acetylation may have an important role in POP pathogenesis.

#### P09.098

#### Analysis of the 8.1 ancestral haplotype and the PAI-1 4G/5G promoter polymorphism in pneumonia-related sepsis

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Sepsis is the main cause of death in the intensive care units, that is initiated by infection and is characterized by a systemic inflammatory response. Numerous family-based studies suggest the importance of genetic factors in the pathophysiology of sepsis.

The acute-phase protein, plasminogen activator inhibitor-1 (PAI-1) is a key element in the inhibition of fibrinolysis and activated protein C. The 4G/5G promoter polymorphism affecting the transcription of PAI-1 has been related to the outcome of sepsis in many studies.

The 8.1 ancestral haplotype (AH8.1) is the most frequent haplotype of the major histocompatibility complex region in the Caucasian population. Our workgroup has recently reported that this haplotype is associated with delayed onset of bacterial colonization in cystic fibrosis.

To evaluate the role of AH8.1 and PAI-1 4G/5G polymorphism in pneumonia-related sepsis, we investigated 207 patients who were treated in the Department of Anesthesia and Intensive Therapy Clinic, Sem-

melweis University. PCR-RFLP and real-time PCR were applied to genotype the PAI-1 4G/5G and five other polymorphisms required to identify the AH8.1.

Our data showed that septic shock was less frequent ( $p=0.092$ ) in carriers of the 8.1 haplotype and significantly less frequent ( $p=0.032$ ) in carriers of the PAI-1 5G allele than in non-carriers. After adjusting for the main clinical parameters a significant protective effect of the AH8.1 was revealed ( $p=0.010$ ). Analysing the two genetic factors together an additive association was found.

These results indicated that the AH8.1 and the PAI-1 5G allele may have protective role in the progression of pneumonia-related sepsis.

#### P09.099

#### The X-linked DIAPH2 gene is a risk factor for Premature Ovarian Failure (POF) involved in actin dynamics of ovarian granulosa cells

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Premature Ovarian Failure (POF) is a complex disorder, resulting in primary or secondary amenorrhea before the age of 40 and affecting 1% of women. A genetic component of the disorder is demonstrated by the prevalence of familial cases. The X-linked DIAPH2 gene was identified as interrupted by the breakpoint of a POF associated translocation. An association study performed in a large cohort of Italian POF patients and controls revealed the presence of a risk-haplotype significantly associated to the disorder. Significant association was also demonstrated in a cohort of German POF thus confirming the role of the gene as risk factor for POF.

The Diaph2 protein belongs to a large family involved in actin cytoskeleton remodeling. We investigated the role of Diaph2 in actin dynamics of human ovarian follicles by eliminating its function by RNAi. We used granulosa cell line (COV434) and primary cells, able to respond to hormonal stimulation. In COV434 expressing Diaph2, actin cytoskeleton forms long filopodia distributed along the cell surface. Diaph2 knock-down caused a strong reduction in the number and in the length of filopodia. In granulosa primary cells, treatment with follicle stimulating hormone (FSH) induced changes in cell morphology and cell-to-cell interactions. This process was abolished when RNAi depleted Diaph2. These results suggest that Diaph2 is involved in actin-mediated cytoskeleton reorganization occurring after hormonal stimulation. Further studies are in progress to elucidate the molecular mechanisms acting in follicle cells in response to FSH stimulation in which Diaph2 could exert its function.

#### P09.100

#### Deletion of LCE3C and LCE3B Genes at PSORS4 Does Not Contribute to Susceptibility to Psoriatic Arthritis in German Patients

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PSORS4 is a susceptibility locus for psoriasis vulgaris (PsV), a common inflammatory, hyperproliferative skin disorder. Recently, a deletion of two late cornified envelope (LCE) genes within epidermal differentiation complex on chromosome 1 was shown to be enriched in 1,426 PsV patients, suggesting compromised barrier function in deletion carriers. We subsequently confirmed this genetic association in a German cohort. In order to investigate whether this variant also predisposes to psoriatic arthritis (PsA), we genotyped this deletion in a case-control cohort of 650 patients and 937 control individuals of German origin. LCE deletion frequency did not significantly differ between PsA patients and controls (65.0 % vs. 65.5 %). Similarly, no evidence for association

to three SNPs in strong linkage disequilibrium with the deletion was observed. This is the first risk factor predisposing only to skin-type of psoriasis supporting the concept of partially overlapping, but different etiological factors underlying skin and joint manifestations.

#### P09.101

#### High-resolution association mapping in the MHC region identifies multiple independent loci for psoriatic arthritis

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Objectives: To identify SNPs in the MHC region which are associated with PsA using a high density map.

Methods: 909 individuals (422 cases and 487 controls) were genotyped for 2299 SNPs in a 5 Mb region on chromosome 6 encompassing the MHC region, using Illumina's MHC Panel Set. The patients were from two well established centers with an expertise in PsA. A stratified case/control analysis was conducted on each SNP separately to test for allelic association with PsA. A Breslow-Day test was used to determine if the odds ratio estimates differed between the two populations.

Results: 17 SNPs from 13 regions were associated with PsA ( $p$ -values  $< 10^{-5}$ ); we report the results for these SNPs, noting their location relative to genes, and their linkage disequilibrium (LD, based on  $r^2$ ) with each other. SNPs rs2734922 (between HLA-H and HLA-A), rs1150735 (between RNF39 and TRIM31), rs11965214 (KIAA1949), rs3130933 (between TCF19 and POU5F1), rs879882 and rs887468 (both between POU5F1 and HGC27), rs2523619 and rs2442719 (both between HLA-C and HLA-B; the latter tags HLA-C\*0802), rs6936035 (between HLA-B and MICA) and rs2516470 (between MICA and HCP5) were associated with PsA, however, none were in strong pairwise LD. In a subset of controls who have also been genotyped for HLA-Cw6, we observed that rs12191877 and rs3906272 are in LD with this allele ( $r^2=0.69$  and 0.60, respectively).

Conclusions: Multiple regions within the MHC are associated with PsA and pairwise LD information suggests that the majority of these signals are independent.

#### P09.102

#### TNF $\alpha$ promoter polymorphisms in psoriatic arthritis patients in Romania

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Background Tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) is a proinflammatory cytokine of critical importance in psoriatic arthritis (PsA) due to its high levels in synovial membrane and fluid and by the efficiency of anti-TNF $\alpha$  treatments in PsA patients [1, 2]. Genetic studies of TNF $\alpha$  polymorphisms in PsA have produced different results that may reflect differences in the ethnic background [3].

Objectives The aim of this study was to investigate two TNF $\alpha$  most studied polymorphisms in PsA patients from Romania.

Methods 45 unrelated well diagnosed patients with PsA (25/20 F/M) and 109 healthy unrelated organ donors (46/63 F/M) were typed for TNF $\alpha$  -308G/A (rs 1800629) and -238G/A (rs 361525) polymorphisms by TaqMan SNP Genotyping Assay C\_7514879\_10 and C\_2215707\_10 respectively (Applied Biosystems, USA).

Results The observed genotypes for the TNF $\alpha$ -308G/A polymorphism (1AA, 6GA, 38GG in cases, respectively 4AA, 24GA, 79GG in controls) and for the TNF $\alpha$ -238G/A polymorphism (0AA, 5GA, 40GG in cases, respectively 0AA, 5GA, 102GG in controls) showed no departure from Hardy-Weinberg equilibrium (HWE). The same results were obtained

when only B27 negative cases and controls were analyzed.

Conclusion The present study shows no departure from HWE in PsA patients in Romania, and no potential association of any investigated allele with the susceptibility to this disease, although a larger number of patients may be required to verify this conclusion.

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#### P09.103

#### Autoimmune-associated LYP-W620 variant reduces T cell activation by altering a reciprocal feedback mechanism

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A missense C1858T single nucleotide polymorphism (SNP) in the PTPN22 gene recently emerged as a major risk factor for human autoimmunity. PTPN22 encodes the lymphoid tyrosine phosphatase LYP, which forms a complex with the kinase Csk and is a critical negative regulator of signaling through the T cell receptor (TCR). The C1858T SNP results in the LYP-R620W variation within the LYP-Csk interaction motif. LYP-W620 exhibits a greatly reduced interaction with Csk and is a gain-of-function inhibitor of signaling. Here we show that LYP constitutively interacts with its substrate Lck in a Csk-dependent manner. TCR-induced phosphorylation of LYP by Lck on an inhibitory tyrosine residue releases tonic inhibition of signaling by LYP. The R620W variation disrupts the interaction between Lck and LYP, leading to reduced phosphorylation of LYP and ultimately to gain-of-function inhibition of T cell signaling.

#### P09.104

#### PTPN22 gene polymorphism in autoimmune endocrine diseases

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Autoimmune destruction of glandular cells is the main reason of type 1 diabetes (T1DM) and Addison's disease (AAD). Both diseases present complex genetic background, with prominent role of predisposing polymorphisms in genes involved in immune reaction. Intriguing candidate is PTPN22 gene, which encodes lymphoid tyrosine phosphatase LYP. Aim of study was to analyse associations of PTPN22 G-(1123)C and C1858T polymorphisms with susceptibility to T1DM and AAD in Polish population. Study comprised 215 T1DM patients (120 females and 95 males) and 87 with AAD (65 females and 22 males), compared to 236 control subjects (133 females and 103 males). Mean age of disease onset was 8.3 ( $\pm 4.3$ ) years for T1DM and 35.2 ( $\pm 11.5$ ) years for AAD. No difference was found in distribution of G-(1123)C polymorphism between T1DM patients and controls, whereas the same analysis in AAD subjects revealed slightly higher frequency of C-(1123) allele ( $p=0.052$ ). C-(1123) allele was significantly more frequent only in affected AAD males, than in controls (OR 2.79; 95%CI 1.39-5.56;  $p=0.003$ ). C1858T polymorphism in PTPN22 gene presented significant association with T1DM (OR 1.73; 95%CI 1.19-2.51;  $p=0.004$ ) and AAD (OR 1.84; 95%CI 1.15-2.94;  $p=0.010$ ). Association of T1858 allele with T1DM remained significant for both genders, while in AAD patients it seemed restricted to male population (OR 2.89; 95%CI 1.24-6.76;  $p=0.011$ ). The haplotype consisting of both mutant alleles, C-(1123)-T1858, was significantly more frequent in T1DM ( $p=0.003$ ) and in AAD ( $p=0.008$ ) than in healthy controls.

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**P09.105****The positive association of +1858C>T polymorphism in PTPN22 gene with type 1 diabetes is replicated in Russian population.**

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The nonsynonymous single nucleotide polymorphism (SNP) +1858C>T within the PTPN22 gene coding the lymphoid tyrosine phosphatase has been associated with type 1 diabetes and other autoimmune diseases such as autoimmune thyroiditis, rheumatoid arthritis and lupus erythematosus. In spite of the low frequency of this polymorphism its association with T1D has been confirmed in several populations. The aim of this study was to replicate type 1 diabetes association with +1858C>T SNP within the PTPN22 gene in Russian population. MATERIAL AND METHODS: the study population included 150 children and adolescents with T1D and 250 unrelated healthy controls. The rs2476601 in PTPN22 (C1858T) was genotyped using restriction fragment length polymorphism. RESULTS: We replicated for the first time in a Russian population the association of the 1858T allele with an increased risk for T1D [ allele T vs. CC: OR (95%) = 1.6 (1.0-2.4); p = 0.002]. CONCLUSION: Our results provide further evidence that the +1858C>T polymorphism is primarily associated with type 1 diabetes and represents a susceptibility factor in Russian population.

**P09.106****Sequencing of candidate genes in Rapid Eye Movement sleep Behaviour Disorder**

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Rapid eye movement (REM) sleep behaviour disorder (RBD) is a sleep disturbance that involves a loss of muscular atonia in the dream-associated REM phase. RBD is known to produce a violent dream-acting-out behaviour and, thus, represents a risk of injury for both RBD affected individuals and their bed partner. The prevalence of RBD is 0.38% in the general population and 0.5% in the elderly population. Several studies have shown that there is an up to 90% male predominance in this disease, which suggests a potential link to chromosome X. To date, no genetic studies have been reported for RBD. Strong evidences lead us to think that there might be a strong relationship between RBD, Parkinson's disease (PD) and Lewy Body Dementia (LBD). We followed this hypothesis to build an interactome of the genes shared by PD and LBD, using experimentally demonstrated human protein-protein interactions available online. Using this interactome as a basis for the mechanism that may underlie RBD, we selected 25 candidate genes, according to different criteria: 1) genes carrying known mutations in either PD or LBD; 2) genes that have strong interactions with genes carrying known mutations in either PD or LBD; 3) genes implicated in other neurodegenerative disorders; and 4) genes located on chromosome X. These candidate genes are currently being screened for mutations in a cohort of 100 RBD patients, which constitutes one of the first attempts to elucidate the genetic mechanisms underlying RBD.

**P09.107****Thrombophilic genes in women with recurrent miscarriage**

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Recurrent miscarriage (RM) is a significant actual problem of modern obstetrics with many etiologies. Thrombophilias is one of the most common cause of RM (40-75%). The contribution of specific inherited thrombophilic genes to this disorder has remained controversial. We carried out the study of polymorphic loci *MTHFR A1298C*, *FGB -455G/A*, *ACE I/D*, *TPA25 I/D* in 4 groups of women with RM: the first group - women with threat of interruption of pregnancy without the burden obstetric anamnesis (n=111), 2 - with spontaneous abortions (n=57), 3 - with the stood pregnancy (n=97), 4 - with the burden obstetric anamnesis (syndrome of loss of a fetus, habitual miscarriage,

stood pregnancy) (n=37), and women with normal physiological pregnancy (n=83). No differences in the frequency of specific gene mutations were detected when women with RM were compared with control women (p>0.05). Only homozygous genotype *DD* of the gene *ACE* was associated with RM (OR=2.67, 95%CI 1.11-6.48, p<0.048). The analysis of intergenic interactions of the studied polymorphic loci revealed 3 two-factorial models of interaction: 1 model consists of two loci *MTHFR A1298C* and *ACE I/D* (p=0.006) and leads to formation of predisposition to spontaneous abortions; 2 - *MTHFR A1298C* and *ACE I/D* (p=0.021), also conducts to the stood pregnancy; 3 - *ACE I/D* and *FGB -455G/A* (p=0.021), and, probably, causes burden obstetric anamnesis. Thus, interaction of studied polymorphic loci *MTHFR A1298C*, *FGB -455G/A* and *ACE I/D* can play the important role in formation of hereditary predisposition to development of thrombophilic conditions at RM.

**P09.108****Genetic variation modifies the effect of ACE-inhibitor treatment: a step towards personalized medication**

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The EUROPA trial demonstrated the efficacy of the ACE-inhibitor perindopril in reducing the incidence of cardiovascular morbidity and mortality. Despite this, wide variation in individual response exists; these differences cannot be accounted for by clinical characteristics. In the current study, interactions between perindopril and 52 SNPs in 12 genes from the renin-angiotensin and bradykinin pathways were analyzed to determine if genetic factors might underlie the variability in treatment response. 8,790 participants from the randomized, double-blinded EUROPA trial with complete phenotypic and follow-up data were genotyped for SNPs selected to ensure coverage of common variation in twelve members of the renin-angiotensin/bradykinin system. Perindopril\*genotype interactions were assessed using multiplicative interaction terms in Cox proportional hazards models. P-values were corrected for multiple tests using permutation. Three perindopril\*SNP interactions, one in the bradykinin receptor B1 gene and two in the angiotensin II type I receptor gene, were significant (H.R. <sub>interaction</sub>[95% C.I.] = 1.44[1.13, 1.83], 1.42[1.09, 1.85], 0.77[0.63, 0.94];  $P_{\text{empirical}} = 0.004, 0.008, 0.011$  and  $P_{\text{permutation}} = 0.012, 0.049, 0.054$ ). Haplotype analysis of these genes provided confirmatory evidence of association. These three SNPs were subsequently typed in a subset of the PROGRESS trial consisting of 1,051 Europeans receiving perindopril treatment. Meta-analysis of the interaction effects from the two populations improved the initially observed P-values and resulted in similar effect estimates for these SNPs (H.R. <sub>interaction</sub>[95% C.I.] = 1.42[1.13, 1.78], 1.41[1.12, 1.78], 0.78[0.65, 0.94];  $P_{\text{meta}} = 0.003, 0.003, 0.010$ ). This study of gene\*medication interaction provides strong evidence that variation in the bradykinin receptor B1 and angiotensin II type I receptor genes modify the treatment benefits of the ACE-inhibitor perindopril and opens new avenues for the successful treatment of hypertension.

**P09.109****SNPs in *CD244* gene associated with rheumatoid arthritis in a Japanese population**

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Rheumatoid arthritis (RA) is a chronic inflammatory disorder characterized by the destruction of multiple joints along with multiple organ involvement. One of the mechanisms of the inflammation in autoimmune diseases associated with signal transduction via signaling lymphocytic activation molecule (SLAM). It was also reported that SLAM family gene, e.g., Ly108 is also associated with systemic lupus erythematosus (SLE). We investigated whether variants of the SLAM family gene in the chromosome 1q region are associated with susceptibility to RA. The association peak in the block was observed at two functional SNPs (rs3766379 and rs6682654) in *CD244* in two independent RA

cohorts from Japan ( $P=3.23 \times 10^{-8}$  and  $P=7.45 \times 10^{-8}$ ). We also found a Japanese cohort of systemic lupus erythematosus (SLE) that had the similar genotype distribution with RA cohorts. These disease-associated SNPs, rs3766379 and rs6682654 have been shown to increase their expression in luciferase and allele-specific transcript quantification assays. Furthermore, we indicated that rs6682654 locates on the binding site of USF-1 in CD244 gene and affect on the regulation of CD244 expression via USF-1. CD244 is a novel genetic risk factor for RA and may have a role for autoimmunity in RA.

#### P09.110

#### Shared genetic susceptibility in primary and secondary restless legs syndrome: A case-control association study in end-stage renal disease patients

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There are two forms of the restless legs syndrome (RLS), primary RLS (pRLS) and secondary RLS. The most common form of secondary RLS is uremic RLS (uRLS) in end-stage renal disease (ESRD). In recent genome-wide association studies (GWAs) we have identified variants (*MEIS1*, *BTBD9*, *PTPRD*, and *MAP2K5/LBXCOR1*) as risk factors for familial and sporadic pRLS. It is not known if the same genetic variants are implicated in uRLS..

We therefore analysed the contribution of the pRLS risk factors to uRLS in a case-control association study in ESRD patients. A total of 642 ESRD patients were scrutinized for symptoms of RLS in face-to-face interviews. 199 were classified as RLS-positive cases (38 familial, 161 sporadic), 443 as RLS-negative controls. Basic dialysis parameters of both groups did not differ significantly (duration of dependence on dialysis,  $P>0.09$ ; average time on dialysis per week,  $P>0.7$ ). We genotyped 10 pRLS-associated SNPs in these groups using the Sequenom iPLEX technology. Statistical analysis was performed using logistic regression with age and sex as covariates. After correction for multiple testing, *MEIS1* (rs12469063,  $P_{corr} = 0.006$ ) and *BTBD9* (rs3923809,  $P_{corr} = 0.002$ ), were associated with uRLS. SNPs in *PTPRD* and *MAP2K5/LBXCOR1* did not show any association ( $P>0.15$ ) with uRLS..

These results support the concept of a partially overlapping genetic predisposition mechanism in pRLS and uRLS.

#### P09.111

#### Polymorphisms in genes with emerging roles in regulation of immune response and their effect on sarcoidosis susceptibility in Slovenian patients

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Sarcoidosis is a chronic inflammatory disease characterised by appearance of granulomas in various organ systems. Among possible causes, genetic factors have been implicated in sarcoidosis aetiology. We have performed the search for novel candidate genes utilising integrative genomics approach, which was based on data from reported transcriptional, proteomic and linkage association studies in sarcoidosis. The search revealed several potential previously uncharacterised candidate genes, among which genes that have recently been implicated in the pathogenesis of inflammatory lung diseases, were selected. Here we present data on association of polymorphisms -1260/ C>A in the *CYP27B1* gene, Lys198Asn in the *EDN1* gene and Ile105Val in the *GSTP1* gene with sarcoidosis in a group of 178 Slovenian patients in comparison with 272 healthy controls. Genotypes were obtained by multiplex polymerase chain reaction using allele specific primers (ASO-PCR). Genotype and allelic frequencies in patients and controls were statistically analysed for association with the disease.

There was no significant association of genotypes or allelic variants of polymorphisms in any of the three genes with susceptibility to sarcoidosis.

Minor effects of polymorphisms in selected candidate genes should be analysed in subsequent studies investigating a larger sample of patients and controls.

#### P09.112

#### Recurrent rearrangements in synaptic and neurodevelopmental genes support the existence of shared biological pathways between schizophrenia, autism and mental retardation

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Comparative genomic hybridization (array-CGH) studies have suggested that rare copy number variations (CNVs) at numerous loci are involved in the aetiology of mental retardation (MR), autism (ASD) and schizophrenia. We have investigated, using QMPSF (Quantitative Multiplex PCR of Short fluorescent Fragments), 28 candidate loci previously identified by array-CGH studies for gene dosage alteration in 247 subjects with MR, 260 with ASD, 236 with schizophrenia or schizoaffective disorder and 236 healthy controls. We show that the collective frequency of CNVs at these loci is significantly increased in autistic patients, patients with schizophrenia and patients with MR as compared with controls ( $p<0.001$ ,  $p=0.01$  and  $p=0.001$  respectively, Fisher exact test). Individual significance ( $p=0.02$ ) was reached for association between autism and a 350 kb deletion located in 22q11 and spanning the PRODH gene. These results support the hypothesis that weakly to moderately recurrent CNVs, either transmitted or occurring de novo, are causing or contributory factors for these diseases. Second, we show that most of these CNVs, which contain genes involved in neurotransmission or synapse formation and maintenance, are present in the 3 pathological conditions, supporting the existence of shared biological pathways between these neurodevelopmental disorders.

#### P09.113

#### Fine mapping of AHI1 on 6q23 as a susceptibility gene to schizophrenia

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Schizophrenia (SCZ) is a multifactorial disorder where probably multiple genes of small to moderate effect act in combination also with environmental factors to increase the risk of illness. In previous studies, using a set of Arab-Israeli families, we identified by linkage and association mapping a susceptibility region to SCZ on the long arm of chromosome 6 (6q23.3); this association was also replicated in an independent sample. The peak region includes the Abelson Helper Integration Site 1 (AHI1) and a putative gene for a human-specific hypothalamic mRNA BC040979.

Here we densely map the most probable candidates in this area, performing a finer mapping of the originally identified linkage peak. The strongest single SNP and haplotype association lies within a 500 kb genomic region (135.6-136.1 Mb) encompassing the AHI1 gene and the BC040979 locus, supporting the role of AHI1 as a susceptibility gene to SCZ. Interestingly the second highest significant subregion is immediately downstream and includes PDE7B and MAP7 genes.

Resequencing of a 10kb region within AHI1 in ethnically defined popu-

lations suggested that the gene has undergone a selective sweep in Europeans, in agreement with previous studies. Network analysis indicated the presence of two haplotype clades, with SCZ-susceptibility haplotypes clustering within the major clade.

#### P09.114

#### Serotonin transporter gene SLC6A4 is associated with negative affect intensity

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Genetic analyses of bipolar disorder (BD) may be facilitated by the use of intermediate phenotypes. We recently showed that euthymic bipolar patients have higher affect intensity than controls (Henry et al. 2008). In the present study, we assessed the 40-items Affect Intensity Measure (AIM) scale (Larsen and Diener, 1986) to 245 bipolar patients and 92 control subjects. As the uni-dimensional structure of the AIM scale has been often questioned, a factorial analysis was performed in the two groups. A similar four factor structure was retained in both samples of bipolar patients and controls: positive affectivity, serenity, negative intensity and negative reactivity. The four dimensions were higher in bipolar patients than in controls ( $p < 0.001$  for positive affectivity,  $p = 0.01$  for serenity,  $p = 0.01$  for negative reactivity and  $p < 0.001$  for negative intensity). Bipolar and controls were subsequently genotyped for the SLC6A4 promoter region insertion/deletion variant (5HTTLPR). Genotypic frequencies were similar in bipolar and in controls ( $p = 0.67$ ). The short allele of the serotonin transporter gene promoter polymorphism was associated with higher negative intensity ( $p = 0.002$ ). The short allele of the SLC6A4 promoter variant may influence the vulnerability to bipolar disorder through its implication in the affect intensity dimension.

#### P09.115

#### Large scale association analysis of SNPs associated with lipid concentrations in 21,010 Japanese individuals

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Recent genome wide association studies identified seven novel loci influencing plasma concentrations of triglycerides, HDL cholesterol and LDL cholesterol in Europeans. For these newly identified loci, large scale replication analyses using other ethnic groups have been awaited. To address this issue, we tested associations between single nucleotide polymorphisms (SNPs) within the seven novel loci and plasma lipid concentrations in 21,010 Japanese individuals. Genotyping of the SNPs was performed by using the TaqMan assay system. Effects of SNP genotypes on plasma lipid concentrations were assessed by using multiple linear regression models including sex, age, BMI, smoking, medication for diabetes as covariates. We observed evidence for strong associations between rs3812316 in *MLXIP* and triglyceride concentrations ( $P \sim 3.0E-11$ , 7.1 mg/dl decrease per minor G allele) and rs599839 in *CELSR2/PSRC1/SORT1* and LDL cholesterol concentrations ( $P \sim 3.1E-11$ , 4.7 mg/dl decrease per minor G allele). SNPs near *ANGPTL3*, *TRIB1* and *GALNT2* showed evidence for associations with triglyceride concentrations ( $3.6E-6 < P < 5.1E-5$ ). The SNP near *TRIB1* also showed association with LDL cholesterol concentrations ( $P \sim 1.2E-5$ ). SNPs in *NCAN/CILP2/PBX4* and *MVK/MMAB* were not associated with any plasma lipid profiles in the Japanese population ( $P > 0.05$ , after Bonferroni correction). The successfully replicated loci are considered to predispose to polygenic dyslipidemia in Japanese. In the case of *NCAN/CILP2/PBX4* and *MVK/MMAB*, ethnic differences both in lifestyle and linkage disequilibrium pattern would contribute to the lack of association in the Japanese population.

#### P09.116

#### Predicting the genetic risk of ischemic stroke: Study of 250 polymorphisms in 1,080 cases and controls

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#### Background:

Environmental and genetic factors contribute to the development of complex diseases such as ischemic stroke (IS), the leading cause of disability and third cause of death in developed countries. In order to identify stroke susceptibility variants, we aimed to study 250 single nucleotide polymorphisms (SNPs), which had been associated with several processes involved in IS such as inflammation, fibrinolysis, coagulation, hypertension, coronary heart disease, angiogenesis, lipid metabolism or diabetes.

#### Methods:

A case-control design was used to analyze 250 SNPs in 183 genes in a Spanish population comprising 270 IS patients with an occlusion in the middle cerebral artery territory and 270 matched controls, free of neurovascular and cardiovascular disorders and familiar history of stroke. The results obtained were replicated in a new similar population of 270 IS patients and 270 healthy controls.

#### Results:

Analysis by additive model revealed 11 SNPs associated with IS. Three of those SNPs were also associated with IS in the replication population, although only two SNPs remained significant after logistic regression analysis adjusting for IS risk factors (age, gender, smoking, hypertension, diabetes mellitus, dyslipidemia): SNP1,  $p = 0.009$ , OR1 = 2.629 (1.279-5.406) and  $p < 0.001$ , OR2 = 3.998 (2.022-7.906); SNP2,  $p = 0.039$ , OR1 = 1.367 (1.016-1.839) and  $p = 0.050$ , OR2 = 1.360 (1.000-1.851), p1 and OR1 corresponding to the first study and p2 and OR2 to the replication study.

#### Conclusion:

We observed an association between two SNPs and an increased risk of IS in the Spanish population. The role of these polymorphisms in IS opens diagnostic and therapeutic expectations and merits further investigation.

#### P09.117

#### Genetic and epigenetic analysis of SSAT gene dysregulation in suicidal behavior

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It has recently been proposed that the SSAT gene plays a role in the predisposition to suicidal behavior. SSAT expression was found to be down-regulated in the brain of suicide completers. In addition, SNP rs6526342 was associated both with variation in SSAT expression and with suicidal behavior. In this study, we aimed to characterize the relationship between SSAT dysregulation and suicide behavior. To this end, we measured SSAT expression levels in the ventral prefrontal cortex (VPFC) of suicide completers ( $n = 20$ ) and controls ( $n = 20$ ) and found them to be significantly down-regulated in suicide victims ( $p = 0.007$ ). To identify the basis of the regulation of SSAT expression, we performed an association analysis of 309 SNPs with SSAT transcript levels in 53 lymphoblastoid cell lines from the CEPH collection. We then examined the methylation status of the SSAT promoter region in suicide completers and control subjects whose SSAT brain expression had been measured. We found no evidence to support a role for SNPs in controlling the level of SSAT expression. SSAT promoter methylation levels were not different between suicide completers and controls and did not correlate with SSAT expression levels. In addition, we found no indication of a genetic association between suicidal behavior and SNPs located within the SSAT gene.

Our study provides new results which show that dysregulation of SSAT expression does play a role in suicide behavior. However, our data do not support any association between rs6526342 and variation in SSAT expression or suicidal behavior.

## P09.118

## Role of interleukin-10, interferon gamma and tumor necrosis factor alpha genes polymorphisms in Suicidal behaviour

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Background: Suicide is a major and growing public health concern throughout the world and yield significant number of morbidity and mortality annually. Some researchers have shown that there is an imbalance between pro-inflammatory and anti-inflammatory cytokines in patient with suicidal behavior. But controversy still is high in this field and further experiments among different population need to be carried out.

Material and methods: To assess whether an association exists between cytokine polymorphisms and suicide risk, 145 patients with suicide attempts, and 160 (150-167) healthy individual genotyped for IL-10, IFN- $\gamma$ , TNF- $\alpha$  polymorphisms using ASO-PCR method.

Results: Our study showed the frequency of IL-10 A/A genotype was significantly higher in the majority of the studied groups in comparison to normal individuals (exception died male vs. normal male (OR, CI: 1.2(0.81, 1.59, 0.61)). IFN- $\gamma$  +874 A/A and A/T genotypes was significantly higher in male bedded vs. male control (OR, CI: 1.9(1.21, 2.59, 0.023)) and male died vs. male control (OR, CI: 1.63(1.22, 2.04, 0.037)), respectively. TNF- $\alpha$  -308 G/G genotype was significantly higher in all studied patients groups vs. normal control, but TNF- $\alpha$  -308 A/A genotype was significantly higher in only Died groups vs. control groups (1.29(1.01, 1.57, 0.047)) and Female died vs. f. control (1.76(1.16, 2.36, 0.014)).

Conclusion: Based on the finding of this study, it is possible to say, IL-10, IFN- $\gamma$  and TNF- $\alpha$  polymorphisms may play a role in suicidal behavior.

## P09.119

## Investigation of complement component C4 loci deficiencies in Russian children with Systemic Lupus Erythematosus (SLE)

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Systemic lupus erythematosus (SLE) is a multisystem, autoimmune inflammatory disease, with both genetic and environmental causative factors. A number of studies have shown that complement component C4 loci (C4A and C4B) are among genes implicated in the pathogenesis of SLE. Patients with C4A deficiency have been previously reported as being susceptible to SLE in different ethnic groups. The aim of our study was to examine the frequencies of homozygous C4A/B deficiency, large deletion of C4A gene and CYP21A pseudogene and 2 bp insertion in exon 29 of C4A/B genes in Russian patients with SLE. The patient group was comprised of 36 unrelated children who fulfilled the American College of Rheumatology classification criteria for SLE. Control group consisted of randomly selected unrelated 76 healthy individuals (50 children and 26 adults). The homozygous deficiency of C4A was in one control child. The homozygous deficiency of C4B was in one patient and in one control adult. The frequency of large deletion of C4A gene and CYP21A pseudogene was 11.11% (4/36) in patients compared to 18.00% (9/50) in control children group, with no significant difference between SLE patients and controls ( $p=0.379$ ). One patient had the 2 bp insertion in exon 29 of C4A gene as well as one control adult. Thus, our results show lack of evidence of a specific role for C4A/B deficiency in determining disease susceptibility among children with SLE in Russian population.

## P09.120

## Evidence for genetic association and interaction between the TYK2 and IRF5 genes in systemic lupus erythematosus

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Objective. Several candidate genes have been implicated in susceptibility for systemic lupus erythematosus (SLE), a complex autoimmune disease. The proposed genes include members of the type I interferon (IFN) pathway and genes involved in immunological defense functions. The aim of this study was to systematically replicate six such genes, TYK2, IRF5, CTLA4, PDCD1, FCGR2A and NOD2.

Methods. Single nucleotide polymorphisms in TYK2, IRF5, CTLA4, PDCD1, FCGR2A and NOD2 were genotyped in 277 SLE patients and 356 healthy controls from Finland, giving a power of 42-70% for different genes at published allele frequencies.

Results. Significant association was seen for rs2304256 ( $p=0.0001$ ) and rs12720270 ( $p=0.0031$ ) in TYK2 and rs10954213 ( $p=0.0043$ ) in IRF5 in our samples, but not for the other genes. We found evidence for genetic interaction ( $p=0.014$ ) between rs2304256 in TYK2 and rs10954213 in IRF5, both members of the type I IFN pathway, further strengthening the role of the type I IFN pathway in SLE pathogenesis.

Conclusion. We conclude that the interferon pathway genes IRF5 and TYK2 may act synergistically in increasing risk for SLE, but our lack of replication does not exclude effects of the other studied genes.

## P09.121

## Classification of low and high risk group of Type 2 Diabetes Mellitus in Maltese and Libyan patients by quantitative expression profiling of SNPlotypes

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T2DM is related to interplay between multiple genes with quantitative effect. Twenty SNPs were studied in the Diabetic patients of Malta and Libya, together with a random number of neonatal controls. Seven SNPs (ADRAB2B [nt46 A->G], FABP2 [codon 54 G->A], UCP1 [nt3826 A->G], LEPTIN [nt -2549 C->A], IPF1 [codon 18 T->C], IL-6 [-174 G->C], TCF7L2 [IVS3 T->G]) were found to be more frequent among Diabetic patients over the reference pool of neonates, and were selected for SNPlototyping analysis, i.e accounting for the total mutant allele number per SNPlotype per person. The log of risk ratio increased with increasing SNP score from -0.7 to 1.08 in Maltese and in Libyan -1.23 to 0.85. The response to Insulin, Metformin and Glibenclamide in T2DM patients representing the different SNPlotypes was measured as a function of differential allele expression on mRNA extracted from monocytes. Only  $\beta$ 2 adrenergic receptor (ADRAB2B) transcripts were detected in the untreated and treated monocytes. The expression of ADRAB2B indicated a relation between the response to the drug and the SNPlotype of the patient. The expression of ADRAB2B was downregulated in response to Insulin and Metformin in patients with SNPlotypes lacking the ADRAB2B mutation and upregulated in the other SNPlotypes. The outcome of ADRAB2B mRNA was dependent on patient SNPlotype, when ranked by wildtype and mutant UCP1 and ADRAB2B polymorphisms. Quantitative mRNA data from selected genes that made up common and rare SNPlotypes, reflect a risk for T2DM due to SNPlotype scoring perhaps useful to classify patients into potentially therapeutic groups.

## P09.122

## The TARGET study: A randomised controlled trial of TPMT testing

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**Background:** Deficiency of thiopurine methyltransferase (TPMT) has been associated with severe neutropenia in individuals treated with thiopurines. Adoption of TPMT testing into clinical practice has been limited by insufficient robust prospective evidence regarding the added value of testing.

**Methods:** A randomized controlled trial was undertaken. Individuals with inflammatory disease requiring azathioprine treatment were randomized (50:50) to receive standard prescribing or TPMT genotyping to inform prescription. The primary endpoint was stopping azathioprine secondary to adverse drug reactions (ADRs). Secondary endpoints included reduction in severe neutropenia, prediction of other ADRs, quality of life and drug efficacy. An economic evaluation was also conducted,

**Results:** Following ethical approval, 333 participants (167 intervention: 166 standard) were recruited from 19 centres in England over 24 months. Overall 111 (33%) patients stopped azathioprine by 4 months due to any ADRs, with no difference in the likelihood of stopping by treatment group (OR 0.91 p=0.71). The incidence of significant neutropenia was lower than anticipated (2.7%). Only one individual (standard arm), was homozygous for the *TPMT\*3A* allele and presented with severe neutropenia. Data on secondary clinical endpoints will be presented.

**Conclusion:** Our findings support the assertion that TPMT testing is specific (individuals homozygous for null alleles are at high risk of neutropenia when treated with azathioprine), but has low sensitivity in detecting individuals at risk of neutropenia. Therefore, TPMT testing is valuable in identifying individuals at risk of neutropenia, but must be performed in tandem with full blood count monitoring to detect individuals at risk of non-TPMT related neutropenia.

#### P09.123

##### Mutation detection of thyroid peroxidase gene in Persian patients with thyroid dyshormonogenesis

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Congenital hypothyroidism (CH) is one of the most common endocrine disorders in childhood. According to morphological findings, CH can be subdivided into defects of thyroid development (dysgenesis), ranging from hypoplasia to athyrosis, and defects of thyroid hormone biosynthesis (dyshormonogenesis) with normal thyroid gland size. The most prevalent cause of dyshormonogenesis is thyroid peroxidase (TPO) gene deficiency. TPO encodes a membrane-bound glycoprotein. This protein acts as an enzyme which plays a central role in iodide organization and is important for thyroid function and pathogenesis. The prevalence of CH is occurring one in 3000-4000 live births in North America and Europe. The recent study in Isfahan province of Iran demonstrated a high frequency of congenital hypothyroidism with 1:370 ratio in newborns. In this study DNA was extracted from peripheral blood of 30 permanent congenital hypothyroidism Persian patients of Isfahan province according to the salting out method. Diagnosis of CH was based on elevated TSH and decreased T4 levels in 3 years old children, who had normal thyroid scan. The 17 exonic region of the TPO gene were amplified and PCR products were analyzed by single strand conformation polymorphism (SSCP) and Sequencing. No exonic mutation in TPO was identified in all patients with dyshormonogenesis CH in this study. It remains possible that these patients' disorder was caused by TPO gene defect in regulatory or intronic region. In addition, other gene defects, such as in the pendrin, thyroid oxidase and thyroglobulin genes, need to be considered and pursued in further studies.

#### P09.124

##### TNF-alpha Gene 308 G/A and 850 C/T Polymorphisms in Turkish Children with PANDAS

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**Objectives:** PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections) is a newly defined pediatric disorder in neuropsychiatry. This disorder occurs with an autoimmune mechanism after Group A Beta Haemolytic Streptococcus infection. Streptococcus infection may cause OCD (Obsessive Compulsive Disorder) or tic disorders and PANDAS. The aim of the current study was to investigate whether there is an association between TNF-alpha gene 308 G/A and 850 C/T polymorphisms and PANDAS.

**Methods:** In this study, 92 cases of PANDAS and 58 control cases were genotyped for TNF-alpha-308 G/A and TNF-alpha-850 C/T polymorphisms with PCR-RFLP method.

**Results:** All 92 PANDAS cases had mutant genotype for 308 G/A polymorphism. In control group, however, cases had 8,62% GA mutant genotype and 91,38% GG genotype.

As for 850 C/T polymorphism, we found 34,78% CC genotype, and 65,22% mutant genotype in PANDAS cases. In control group, however, 46,55% mutant genotype and 53,45% CC genotype was observed.

In our study, we observed remarkable effects of these two polymorphisms particularly on phenotype-genotype relations. In different parts of the world, studies conducted for different OCD patients have variable results. Moreover, there have not been any study revealing an association between PANDAS and TNF-alpha gene polymorphisms reported yet.

**Conclusion:** Here, we have demonstrated an association between TNF-alpha 308 G/A and 850 C/T polymorphisms and PANDAS susceptibility. In light of our findings, we propose that these two polymorphisms can be used as a molecular indicators of PANDAS which needs to be verified by further research on PANDAS.

#### P09.125

##### Association of candidate genes for type 1 diabetes with parameters of vasoactive and proteolytic systems

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Associations of polymorphisms in genes *NOS1* (C3392T), *NOS3* (C-691T, VNTR, C774T, G894T), *TNFA* (G-308A), *IL1A* (+3953 A1/A2), *IL1RN* (VNTR), *IL4* (G717C) and *IL4RA* (A148G) with parameters of vasoactive and proteolytic systems at children and the adolescents with type 1 diabetes (T1D) were studied. 119 children and the adolescents with T1D have been surveyed. Biochemical research included definition of: kallikrein (KK), kallikreinogen (KKG), angiotensin converting enzyme (ACE),  $\alpha$ 1-proteinase inhibitor ( $\alpha$ 1-PI),  $\alpha$ 2-macroglobulin ( $\alpha$ 2-MG). At T1D group, there was an increase in activity of KK, and development of diabetic neuropathy (DN) was accompanied by hyperactivity of ACE and decrease in activity of  $\alpha$ 1-PI in blood. Children and adolescents who were carriers of C allele of *C774T* (*NOS3*) had lower values of KKG than TT homozygotes ( $p=0,043$ ). Carriers of G allele of *G894T* (*NOS3*) had lower values of  $\alpha$ 1-PI than TT homozygotes ( $p=0,008$ ). For polymorphism *A148G* in *IL4RA* we found that activity of ACE was increased in carriers of A allele ( $p=0,008$ ). The obtained associations of investigated polymorphisms with parameters of vasoactive and proteolytic systems confirm participation of these genes in development of endothelial dysfunction at diabetes.

#### P09.126

##### "Genetic Load" of diabetes risk alleles affects beta-cell function in patients with newly diagnosed type 2 diabetes

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Genetic variation in a number of SNPs has been demonstrated to affect the risk of type 2 diabetes mostly by genome wide association studies. Some of these "diabetoSNPs" have been associated to prediabetic phenotypes in nondiabetic individuals. However, no studies have re-

ported associations between "diabetoSNPs" and the patient metabolic phenotype. We assessed beta-cell function and insulin sensitivity in 343 consecutive GADA-negative, drug treatment naive patients with newly diagnosed type 2 diabetes by state-of-art methods. Both beta-cell function and insulin sensitivity of these patients were severely reduced (~60%,  $p<0.001$ ) when compared to healthy controls.

In all patients alleles of rs7903146 (TCF7L2), rs5219 (KCNJ11), rs10946398 (CDKAL1), rs1111875 (HHEX2), rs679931 (CACNA1E), rs1801282 (PPAR-gamma) and rs1044498 (ENPP1) were determined. According to the number of the risk alleles, the patients were divided in 3 groups: "low" ( $<=6$  alleles), "intermediate" (7-8 alleles), "high" ( $>8$  alleles) genetic load. No differences in insulin sensitivity were detected among the 3 groups. However, the dose-response curve relating glycemia to insulin secretion rate was significantly lower in the "intermediate" and "high" genetic load groups (Delta: ~30% in both groups,  $p<0.02$ ) than in the low genetic load group, to indicate a more severe defect in beta-cell function in patients burdened with a higher genetic risk of the disease. In newly diagnosed type 2 diabetic patients a low genetic load hallmarks patients with better beta-cell function. Our data imply that the ever expanding number of "diabetoSNPs" might be useful to assess the metabolic phenotype of the patients.

#### P09.127

##### The transcription factor 7-like 2 (TCF7L2) gene variants and risk of Type 2 diabetes

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Although type 2 diabetes mellitus has a strong genetic basis, until recently, most candidate genes for type 2 diabetes mellitus (T2D) have shown only modest effects and the associations have been inconsistent. Recent studies have implicated variants of the transcription factor 7-like 2 (TCF7L2) gene in genetic susceptibility to T2D in several different populations. The TCF7L2 gene is a member of the T-cell factor (TCF)/lymphoidenhancing factor family of high mobility group box-containing transcription factors involved in the Wnt signaling pathway. This pathway is a key component to the regulation of cell proliferation and differentiation. The aim of this study was to determine whether variants of this gene are also risk factors for T2D developments in northwest of Iran (Azerbaijan). We genotyped two of these polymorphisms (rs12255372 and rs7903146) in an admixed sample of 100 patients with T2D and 100 controls. Study were performed using polymerase chain reaction-restriction fragment length polymorphism assay to investigate the association of rs12255372 (G/T) and rs7903146 (C/T) polymorphisms of the TCF7L2 gene with T2D. These variants in TCF7L2 seem to be associated with an increased risk of diabetes among persons with impaired glucose tolerance. The risk-conferring genotypes in TCF7L2 are associated with impaired beta-cell function but not with insulin resistance. These data suggest that inherited or acquired changes in TCF7L2 expression or function are mechanistically involved in causing type 2 diabetes mellitus.

#### P09.128

##### Association of FokI vitamin D receptor gene polymorphisms with psychiatric disorders

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Introduction: Low level of vitamin D is known to alter brain development, and is a candidate risk factor for psychiatric disorders. This study examines the association of FokI polymorphisms in the vitamin D receptor (VDR) with schizophrenia, bipolar mood disorder (BMD).

Methods: In a case-control study 43 Schizophrenic, 73 bipolar mood disorder patients and 100 healthy controls were recruited. All psychiatric disorders were diagnosed according to DSMIV criteria. Healthy controls had no familial history of these disorders. DNA extraction was performed from whole blood. Vitamin D receptor gene polymorphism (FOKI) was genotyped by PCR-RFLP method.

Results: The frequency of FF, Ff, ff genotypes in healthy controls were 54 %, 39% and 7% respectively. These frequencies were 58.13%,

34.8% and 6.97% in schizophrenic patients and 69.86%, 19.17% and 10.95% in BMD patients. The homozygote FF genotype was more common in BMD patients compare to healthy controls ( $p=0.04$ ). The odds ratio between frequency of FF genotype in BMD and healthy control was 1.97 with 95% confidence interval was between 1.04 to 3.73. There was no significant association between FF genotype and schizophrenia ( $p=0.7$ ).

Conclusion: Previous studies indicated a possible biochemical mechanism occurring between vitamin D and mood disorders. Since VDR FOKI polymorphism has been found to correlate with serum vitamin D concentrations, it was expected this gene variation associate with bipolar mood disorder as we found. Also VDR as a nuclear receptor may contribute to psychiatric disorders via modifying of neurotransmitters transcriptions like dopamine and serotonin.

#### P09.129

##### Association of gene polymorphisms with power performance in wrestlers

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Analyses of the genetic determinants of strength and power provide information concerning the contribution of both genes and environmental factors. We have recently shown that rare PPARG (peroxisome proliferator-activated receptor gamma) 12Ala and PGC1B (PPARgamma coactivator-1-beta) 203Pro alleles are overrepresented in elite power-oriented athletes compared to controls. PPARGamma is involved in regulation of insulin pathway; whilst PGC1b coactivates the MEF2 family of transcription factors to stimulate the type IIX myosin heavy chain promoter (IIX MHC determines fast glycolytic muscle fiber phenotype). Accordingly, the aim of the study was to investigate gene polymorphisms for association with power performance in Russian elite male sambo wrestlers. Determination of knee extensor peak power and peak movement velocity were performed using dynamometer Bidex. PPARG Pro12Ala and PGC1B Ala203Pro gene polymorphisms were determined by PCR-RLFP. We found that PPARG 12Ala allele was significantly associated with increased relative knee extensor peak velocity of athletes (Ala/Ala - 694 deg/sec/kg (%), Pro/Ala - 609 (66) deg/sec/kg (%), Pro/Pro - 495 (84) deg/sec/kg (%);  $P=0.03$ ). Furthermore, carriers of PGC1B 203Pro allele exhibited the highest values of average knee extensor peak power (Ala/Ala - 250 (57) W, Ala/Pro - 393 (42) W;  $P=0.014$ ). In conclusion, PPARG Pro12Ala and PGC1B Ala203Pro gene polymorphisms are associated with power performance in sambo wrestlers.

#### P09.130

##### Analysis of MTR A2756G and MTRR A66G polymorphisms among mothers of CL/P children from West Ukraine

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Craniofacial anomalies, particularly cleft lip/palate (CL/P), are among the most common birth defects. Polymorphisms in genes encoding enzymes involved in the metabolism of homocysteine, such as methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR) and methionine synthase reductase (MTRR) could play a role in the mechanisms predisposing for clefting.

Contradictory findings have been recently published on the evaluation of the mutant allele: formerly called MTRR A66G, but now called MTRR G66A, because the G allele was found to be more common than the A allele based on the new data. There are considerable differences in frequencies of genotypes between different population and ethnic groups.

DNA of mothers of CL/P children's were analyzed for chosen polymorphism MTR 2756AG and MTRR 66AG by PCR-RFLP assay.

The analysis of 2756AG of the MTR gene showed that the GG genotype was more prevalent in the mother group (0.22) as compared to controls (0.12), G allele frequencies were - 0.36 and - 0.30 among the cases and controls, respectively, and these differences were not statistically significant.

The analysis of 66AG polymorphism of MTRR gene revealed high frequency of 66G allele: 0.61 and 0.78 in studied and in the control

groups respectively. The heterozygous genotype 66AG of MTRR gene was more prevalent among the mother of CL/P children's (55%) as compared to controls (36%). Only this difference was statistically significant ( $p<0.05$ ).

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## P10. Evolutionary and population genetics, and Genetic epidemiology

### P10.01

#### Genetic risk factors for cardiovascular diseases in the Roma minority population of Croatia

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The Roma, formed as a founder population that has undergone multiple consecutive bottlenecks and consequently experienced genetic drift together with its persistent socially, politically and culturally determined isolation, present almost ideal target population for investigation of genetic factors influencing development of complex diseases. In this investigation we assumed that Bayash Roma population living in Croatia could be exemplary for having population-specific allele frequencies, including those for candidate genes for cardiovascular disease. To assess the genetic risk factors for development of cardiovascular disease in the population of Bayash Roma we investigated the allele frequencies of the following polymorphisms: ACE I/D, eNOS VNTR and LEP G-2548A. The allele frequencies in a sample of 230 Bayash Roma of Croatia are compared with the data reported for other European and world populations and the geographic distributions of minor allele frequencies are presented. Population analysis showed that the Bayash Roma population living in Croatia has typical European frequency of the I allele of the ACE I/D polymorphism (45%), but the frequency of the allele 4 of the eNOS VNTR polymorphism (12%) as well as of allele G of the LEP G-2548A (34%) are lower than those found in surrounding European populations placing Croatian Roma population further east, among Asian populations. Present analysis showed that the Bayash Roma of Croatia in comparison with other European populations, do not carry increased genetic risk for cardiovascular diseases related to the most common polymorphisms of the ACE, eNOS and LEP genes.

### P10.02

#### Highest prevalence of Alpha-1-antitrypsin deficiency found in Madeira Island

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Alpha-1-antitrypsin (AAT) deficiency is a common genetic disease that affects lung and liver. Early diagnosis in asymptomatic patients helps on modifying lifestyle choices and reduces the risk of Chronic Obstructive Pulmonary Disease (COPD). The determination of the prevalence of this genetic deficiency on Madeira Island (Portugal) population is important to clarify their susceptibility and define the importance on a general application of genetic tests for AAT on populace at risk of COPD.

Two hundred unrelated samples from Madeira Island were genotyped for the two most common AAT deficiency alleles, PI\*S and PI\*Z, using Polymerase Chain Reaction - Mediated Site-Directed Mutagenesis. Results show one of the highest frequencies found worldwide for both mutations. In fact, PI\*S mutation in Madeira Island population (18%) have the highest prevalence comparing to any world population already studied, and PI\*Z mutation (2.5%) was the third higher found worldwide. The frequency of AAT deficiency genotypes in Madeira (PI\*ZZ, PI\*SS and PI\*SZ) was estimated to be the highest studied: 41 per 1000.

This high prevalence of AAT deficiency on Madeira Island reveals an increased genetic susceptibility to COPD and recommends a routine genetic testing for people at risk.

### P10.03

#### Genome-wide association study in late-onset Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative disorder that affects more than 5% of the individuals older than 65 years. Apolipoprotein E (APOE), the universally confirmed risk gene for late-onset Alzheimer's disease (LOAD), is neither necessary nor sufficient to cause AD, and 50% of the genetic-risk effect of the disease still remains unexplained. As an attempt to discover new markers associated to LOAD, we are carrying out a multi-stage case-control genome-wide association study, an approach that has the potential to effectively identify genetic contributions to complex diseases. About 400 individuals were sampled and genotyped for 312,000 single nucleotide polymorphisms (SNPs) using the *Infinium* technology of Illumina Inc. Additionally, PCR-RFLP based APOE genotyping was used to confirm significant association of this gene with LOAD in the studied cohort. Logistic regression with age, sex and APOE genotype as covariates was performed on the 375 individuals (191 cases and 185 controls) and 302324 SNPs that fulfilled established quality-control criteria. There are some interesting chromosomal regions in which there are several markers with low p-values, although in this first stage no SNP showed statistically significant association with the disease after applying the corrections for multiple testing. Potential association of copy-number variations with LOAD is also being studied. These data, together with functional annotation of the likely associated SNPs, will guide us in the selection of markers to be genotyped in a more statistically powerful second-stage that will be performed in an independent sample set.

### P10.04

#### Analysis of CFH, LOC387715, HTRA1 polymorphisms and ApoE alleles with susceptibility to age-related macular degeneration in Hungary

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Introduction: Age-related macular degeneration (AMD) is a leading cause of irreversible central vision loss in the elderly worldwide and its prevalence increases with age. AMD has a strong genetic component. The goal of our study was to establish the frequency of Tyr402His polymorphism of the CFH gene, rs10490924 polymorphism at LOC387715, rs11200638 polymorphism of the HTRA1 gene and different ApoE alleles in Hungarian AMD patients and to determine disease risk conferred by these factors.

Methods: 105 AMD patients and 95 unrelated healthy controls were analyzed in our case-control study. According to disease severity, 48 patients were assigned to the early and 57 patients to the late AMD subgroup. The mutations were tested using PCR-RFLP.

Results: When hetero- and homozygous mutation carriers were combined and tested against wild type individuals, CFH, LOC387715 and HTRA1 odds ratios were 1.8 (95%CI:1.0-3.3), 2.0 (95%CI:1.1-3.6) and 2.2 (95%CI:1.2-4.0), respectively. In the early AMD subgroup, homozygous CFH, LOC387715 or HTRA1 polymorphisms conferred 4.9-fold (95%CI: 1.7-14.2), 7.4-fold (95%CI: 2.1-26.2) or 10.1-fold (95%CI: 2.5-40.8) greater likelihood of disease, respectively. In the late AMD subgroup, homozygosity for CFH, LOC387715 or HTRA1 risk alleles resulted in 10.7-fold (95%CI: 3.7-31.0), 11.3-fold (95%CI: 3.2-40.4) or 13.5-fold (95%CI: 3.3-55.4) increased disease risk, respectively. No association was found between ApoE alleles and AMD in our groups. Conclusions: The analyzed CFH, LOC387715 and HTRA1 polymorphisms strongly associate to the development of AMD in the Hungarian population. The association is particularly strong when homozygous risk alleles are present and in late stage of the disease.

### P10.05

#### Capturing the genetic variation in the ATGL gene by Ecotilling: the rare mutation approach

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**Background:** Several recent studies highlight that especially rare mutations exhibit a strong influence on many atherosclerosis-related phenotypes. Since the ATGL lipase catalyzes the rate-limiting step of the lipolysis, it represents an important candidate gene for the investigation of the influence of rare mutations on the lipid metabolism.

**Aim of the study:** To capture the entire genetic variation of ATGL in a large healthy working population with a special focus on potentially regulatory non-coding regions.

**Methods:** The full ATGL gene region including the predicted promoter was screened for mutations in 1473 individuals of the SAPHIR study by using a pooled Ecotilling approach. All polymorphisms were confirmed by sequencing, bioinformatically characterized and four common SNPs (2 of them being previously unknown) were finally genotyped in SAPHIR and in the Utah obesity case-control study.

**Results:** We detected 58 new, previously unknown mutations: 33 private mutations, 20 SNPs with a frequency below 5% and 5 common polymorphisms. Eleven mutations affected the protein sequence and ~30% of all detected variants were located in the 5' upstream region. Interestingly, only 16 of the 25 reported dbSNPs could be confirmed. Association studies with phenotypes from lipoprotein metabolism are currently ongoing.

**Conclusion:** Rare mutations are relatively common and may represent an important factor for the diversity of lipid-related phenotypes in a population. We found several new mutations to be located in potentially functional sites such as exons and promoters. This highlights the current lack, respectively need, of comprehensive information about the distribution and impact of rare genetic variants.

#### P10.06

#### APOA5 gene polymorphism and triglyceride levels in metabolic syndrome patients

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Metabolic syndrome (MS) is a clustering of abdominal obesity, increased triglycerides, low levels of high density lipoprotein cholesterol, high blood pressure, and elevated fasting glucose levels and consists of multiple risk factors that are increasing the cardiovascular mortality. Naturally occurring variants of the apolipoprotein A5 gene have been associated with increased triglyceride level and have been found to confer risk for cardiovascular diseases. In our study four haplotype-tagging polymorphisms, the T-1131C, IVS3+G476A, T1259C, and C56G alleles were analyzed. A total of 325 metabolic syndrome patients were genotyped by polymerase chain reaction - restriction fragment length polymorphism. MS patients were separated into four quartile (q) groups based on triglyceride levels (q1: TG<1.38 mmol/l; q2: 1.38-1.93 mmol/l; q3: 1.94-2.83 mmol/l; q4: TG>2.83 mmol/l). We observed significant relationships between three APOA5 minor allele carrier frequencies and plasma triglyceride quartiles: -1131C (q1: 4.94%; q2: 8.64%; q3: 11.6%; q4: 12.3%), IVS3+G476A (q1: 4.32%; q2: 7.4%; q3: 10.36%; q4: 11.1%), 1259C (q1: 4.94%; q2: 7.41%; q3: 10.4%; q4: 11.7%). The serum total cholesterol levels did not show allele-dependent differences. The findings presented here revealed unique arrangement of APOA5 minor alleles in MS.

#### P10.07

#### Determination of genetic profiles associated with atopic asthma in Madeira population

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Atopic asthma arises from gene-environment interactions. There are many genes associated to asthma and their combination may enlighten some results obtained in other studies regarding different populations.

A sample of 100 children with atopic asthma was compared with 105

individuals from Madeira Island population. Five different polymorphisms with proven association to the asthmatic disease development were studied, namely IL4-590 C/T (rs2243250), ADAM33 S1 G/A (rs3918396), GSDML C/T (rs7216389), STAT-6 C/T (rs324011) and IL13 +2044 G/A (rs20541). Genotyping was performed by Real Time PCR. Allelic and genotypic frequencies were determined for each polymorphism. We also determined the genetic profile of each individual by combining the five polymorphisms. Comparisons between both populations were done using  $\chi^2$  test for each polymorphism and the Fisher's test to compare all genetic profiles. Significant differences have been found amongst studied populations for IL4-590 (C/T) ( $p<0.05$ ) opposite to the remaining polymorphisms. The overall genotypic profile combination revealed the presence of specific profiles on the patients' sample which are absent on Madeira's population.

By increasing the number of studied polymorphisms new specific asthma predisposing profiles may be identified. The definition of asthma risk-associated profiles will allow an early intervention in terms of primary prevention directed towards children in these families.

#### P10.08

#### Mutational Spectrum of Congenital Adrenal Hyperplasia in Greek Cypriot Patients

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Congenital adrenal hyperplasia (CAH) is a common autosomal recessive disorder mainly caused by defects in the steroid 21-hydroxylase (CYP21) gene. 21-hydroxylase deficiency (21-OHD) leads to impaired synthesis of cortisol from cholesterol by the adrenal cortex. In 21-OHD CAH, excessive adrenal androgen biosynthesis results in virilization in all individuals and salt wasting in some individuals. More than 90 % of cases of CAH are caused by point mutations or deletions of the CYP21 gene which they are attributable to conversion of DNA sequences from its neighbouring duplicated CYP21P gene. The aim of our study was to determine the mutational spectrum of CYP21 and the genotype and phenotype correlation in Greek Cypriot patients with CAH. Molecular analysis was performed in 41 CAH patients and 99 family members. The most frequent genetic defects were V281L (50.68 %) in the non-classical form and 8bpΔE3 (20.54 %) and I<sub>2</sub> splice (12.32 %) in the salt wasting form. Compared with other populations, Greek Cypriot patients had a higher frequency of V304M missense mutation (4.10 %) in the nonclassical form. The severity of the genetic defects and the clinical-laboratory features of our patients are well correlated. Thus, these results underline the importance of genetic evaluation and counselling in hyperandrogenic women who are predicted to carry CYP21 causing mutations by biochemical tests. There is also evidence that the heterozygous carrier state for CYP21 mutations can be associated with symptoms of androgen excess in certain susceptible individuals.

#### P10.09

#### Geographical endogamy in Morocco

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This study aims to evaluate the geographical marital endogamy among the population of Rabat-Salé-Zemmour-Zaer region in Morocco in order to estimate the reproductive isolation (or openness) of the population studied.

The study was conducted within a randomly selected sample of mothers postpartum in Souissi maternity in Rabat city, between November 2004 and June 2005. Endogamy among 270 couples was compared to that seen among their parents. Various types of endogamy were measured, based on the place of birth, place of residence and geographical origin of the spouses and their parents. The results show the tendency of couples toward geographical endogamy. This tendency is very strong among couples within parental generation. The results of intergenerational comparisons can be concluded to a significant decrease of geographical endogamy from one generation to the next (parents and children) ( $p<0.001$ ). The method homogamy index confirms this decrease. However, the values obtained do not exclude the importance of this marital behavior among the younger generation.

## P10.10

## Catechol-O-methyltransferase gene polymorphism study in uterine leiomyoma patients from Russia

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Catechol-O-methyltransferase (COMT) is one of several enzymes participating in catecholamines metabolism. The gene COMT (22q11) contains a G-to-A transition polymorphism in codon 158, which results in a valine-to-methionine substitution in its protein product. Several studies postulate association of this polymorphism with uterine leiomyoma - a common, benign, smooth muscle tumor of great medical and social significance. The aim of our study was to investigate possible association of COMT G158A polymorphism with uterine leiomyoma in women from North-West of Russia. DNA was extracted from blood samples of 33 uterine leiomyoma patients and from 68 control females and subjected to PCR-RFLP analysis. The relative frequencies of AA, AG, GG genotypes were 0.429, 0.257 and 0.314 in the uterine leiomyoma group, respectively, compared to control subjects - 0.426, 0.176 and 0.398, respectively. No statistically significant differences with respect to allele frequency and genotype distribution were ascertained for COMT G158A polymorphism in the patients and compared to the control groups ( $P=0.6$  and  $P=0.6$ , respectively). Thus far we could not confirm association of the COMT G158A polymorphism with risk of uterine leiomyoma in women from North-West of Russia.

## P10.11

## Prevalence of consanguineous marriage in Afyonkarahisar and its relation with the occurrence of congenital anomalies

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This study was performed to search the frequency and the causes of consanguineous marriages and their effects on spontaneous abortions and births with congenital abnormalities. In this study, only one person was selected from each family by random sampling method and face to face survey method was performed. Consanguineous marriage was detected in 381 (19.6%) of 1940 married people in the families studied. It is detected that first cousin marriage was the most frequent one with 14.8% and the second was other cousin marriages with 4.8% frequency. When first marriage age was evaluated, especially the frequency of consanguineous marriage was detected to be high at the ages of 17 and less, while it was rare at the ages of 31 and over. It was detected that consanguinity between parents and low education level increased the frequency of spontaneous abortion and congenital abnormalities. The frequency of spontaneous abortion and congenital abnormalities in families with consanguineous marriage was found to be significantly higher than the group made foreign marriage.

Consanguineous marriage and its degree in Afyonkarahisar		
Consanguinity / Degree	n	%
No	1559	80.4
Yes (First cousin marriage)	287	14.8
Yes (Second cousin marriage)	94	4.8
Total consanguineous marriage	381	19.6
Total number of population	1940	19.6

## P10.12

## Assessment of a relationship between consanguinity and early pregnancy loss in Tunisian population

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In this study, we've tried to establish the possible relationship between recurrent miscarriage and consanguinity in the Tunisian population, where the prevalence of first cousin marriage is about 50%. A cluster sample of 100 married couples, representative of all population groups and all geographic locations of Tunisia were randomly selected whom were asked whether or not they had experienced a stillbirth or

a spontaneous abortion. The consanguineous women of early pregnancy losses were compared with non-consanguineous women from the same population and with the same obstetrical history, matched for maternal age. The investigation showed no difference in the rate of maternal disorders. There was also no evidence of familial clustering of recurrent miscarriage in both groups. The absence of a relationship between recurrent miscarriage and consanguinity in Tunisia could be due to the particular characteristics of the native Tunisian population, in which rare recessive genes are uncommon, or overall to the absence of an association between recurrent miscarriage and consanguinity.

## P10.13

## Polymorphisms of Genes involved in oxidative stress response PON1 (Q192R, L55M), Mn-SOD (Ala16Val) and CAT (C-262T) and the Development of Coronary Artery Disease (CAD) in Patients of Different Age and Sex in St. Petersburg, Russia.

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The higher levels of lipid peroxidation products in CAD patients could be related to a higher susceptibility of their plasma lipoproteins to oxidation and/or to a decrease of plasma antioxidant defenses.

We have investigated the associations of the SNPs of PON1 (Q192R, L55M), Mn-SOD (Ala16Val) and CAT (C-262T) genes involved in oxidative stress response with levels of total CH, LDL-CH and HDL-CH. Materials: 228 men, survived myocardial infarction (MI) under the 45 (group I), 95 men with MI after 60 years (group II) and 115 healthy men (group III); 74 angiographically diagnosed CAD women (group IV) and 85 women after 80 years without CAD (group V).

Genotypes were determined by PCR-RFLP.

The QR genotype of PON1 192 was more frequent in group I compared to group IV ( $p=0.001$ ), the frequency of MM genotype of PON1 55 were significantly lower in group V compared to group I ( $p=0.019$ ). Total cholesterol level was higher in QR patient, than in QQ and RR carriers of group V ( $p=0.026$ ).

There were no differences in genotype distribution of CAT C-262T among our groups. But, T/T carriers of CAT had lower level of HDL-CH in group IV ( $p=0.005$ ).

The Val/Val genotype of Mn-SOD was more frequent in group III than in group I ( $p=0.013$ ). Concentration of LDL-CH in Val/Val genotype carriers was lower compared to Ala/Ala or Ala/Val carriers ( $p=0.004$ ) in group I.

The genetic variants of PON1, Mn-SOD and CAT are associated with lipoprotein levels in CAD patients in view of their sex and age.

## P10.14

## Investigation of COX-2 -765G→C promoter variants among Iranian and Iraqi populations

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Cyclooxygenases-2 enzyme (COX-2) elevates in chronically inflamed tissues. It converts arachidonic acid to prostaglandins. It has been shown that the COX-2 -765G→C promoter polymorphism is associated with decreased promoter activity, which results in decreased COX-2 expression and the response to non-steroidal anti-inflammatory drugs (NSAIDs). In addition, it has been reported that this polymorphism is associated with increased risks of several types of cancers and inflammatory diseases.

The aim of the study is to determine the prevalence of the COX2 gene polymorphism in different ethnic groups in Iran compared with those from Baghdad city in Iraq. The study population was selected from eight cities. After obtaining signed informed consents, blood samples were collected. Genotyping was performed with PCR-RFLP and confirmed with sequencing. Range of the C allele frequency was 11.4%

in Tehran to 27.1% in Shiraz. Overall, there were no significant differences in allele frequencies across cities or ethnic groups. However, the allele frequency of the COX-2 genotype in the Tehran population was significantly different from that of the Shiraz population (p value = 0.048). The results indicated that the prevalence of this COX2 polymorphism did not differ in healthy populations from Iran and Baghdad. Thus, this polymorphism may be a suitable target for genetic research in the field of inflammatory diseases among Iranian and Arab populations.

#### P10.15

#### Study of association between polymorphism of 5 genes and Crohn's disease in Russian population

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Crohn's disease (CD) is a chronic relapsing inflammatory bowel disorder which etiology remains unknown. At present the incidence of this disorder increases in the world. Therefore CD is thought to be one of the serious problems in the gastroenterology.

Allele and genotype frequencies of 8 polymorphic variants of 5 genes such as *NOD2/CARD15* (Arg702Trp, Gly908Arg, Leu3020InsC), *TNFA* (-238G/A, -308G/A), *VDR* (Taq-1), *IL-4* (C-590T), *IL-4R* (Q576R) were studied in CD patients (102 individuals) and in controls (112 individuals). The patients and the control individuals were recruited in the North-West region of Russian Federation. The frequency of 3020<sup>l</sup>nsC allele of *NOD2/CARD15* gene was 3-fold higher in CD patients compared to controls (18% against 6%, p=0.006). In patients with complicated forms of CD the 3020<sup>l</sup>nsC allele was significantly more frequent (27%) compared to patients with inflammatory forms of CD (8.3%; p=0.02). 26.5% of CD patients were carriers of the rare allele -308<sup>A</sup> for -308G/A polymorphism of *TNFA* gene and only 8% of controls (p=0.0004). The frequency of heterozygote genotype -308<sup>GA</sup> was significantly higher in CD patients than in controls (24.5% against 8%, p=0.0004). Frequency of 576<sup>RR</sup> genotype of *IL-4R* gene was significantly higher in CD patients (11.7%) in comparison with controls (1.4%; p=0.016, OR=9; 95% CI:1.15-71.47).

Thus, 3020<sup>l</sup>nsC, -308<sup>A</sup> alleles and 576<sup>RR</sup> genotype could be the risk factors of CD in Russian population. We suggest that DNA-testing of polymorphic variants of *NOD2/CARD15*, *TNFA*, *IL-4R* genes is important for the presymptomatic diagnostics of Crohn's disease.

#### P10.16

#### CYP2C19\*2, CYP2C19\*3 and CYP2C19\*4 alleles frequencies in a Romanian population

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CYP2C19, a member of the cytochrome P450 superfamily, metabolizes around 15% of the clinical relevant drugs. Its coding gene, *CYP2C19*, has been shown to be polymorphic, two prominent alleles, *CYP2C19\*2* and *CYP2C19\*3*, well studied in many populations, being associated with a poor metabolizer phenotype. The *CYP2C19\*4* allele, which is also associated with a poor metabolizer status is less characterized in the Caucasian populations. Taking into consideration the lack of data regarding the frequencies of these three *CYP2C19* alleles in Romania, the aim of this study was to provide a first evaluation of these alleles on a Romanian population group. The *CYP2C19* alleles were studied in 200 healthy unrelated individuals. PCR-RFLP assays were employed for the study of each *CYP2C19* allele, plus an extra tetra-primer PCR assay used to randomly verify the results obtained with PCR-RFLP for *CYP2C19\*2* and *CYP2C19\*3* alleles. *CYP2C19\*2* was observed in heterozygous state in 49 individuals (24.5%) and in homozygous state in 3 individuals (1.5%), while *CYP2C19\*3* allele was not demonstrated in any individuals. *CYP2C19\*4* was seen in heterozygous state in one individual (0.5%), who was heterozygote for *CYP2C19\*2* as well. Thus, the allele frequencies for *CYP2C19\*2*, *CYP2C19\*3* and *CYP2C19\*4* were 13.75%, 0% and 0.25%, respectively. Overall, 4 individuals (2%) included in this study are predicted to be *CYP2C19* poor metabolizers. Genotyping for *CYP2C19* variants before starting the medication with drugs substrates for *CYP2C19* could reduce the impact of adverse drug reactions and treatment failures, based on an

individual, optimized drug therapy.

#### P10.17

#### Study of DFNB59 gene mutations in exon 2 and 4 in association with deafness using PCR-RFLP in a Province of Iran

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**Background and aim:** Hearing loss is a heterogeneous disorder and may be due to genetic or environmental cause. A novel gene, DFNB59 encodes pejvakin has been very recently shown to cause neural deafness. This study aims to determine the frequency of DFNB59 gene mutations in exon 2 and 4 in 100 patients negative for GJB2 mutations in a province of Iran.

**Methods:** In this descriptive-lab based study we investigated the frequency of DFNB59 gene mutations in exon 2 and 4 of the gene.

DNA was extracted from all patients following the standard phenol chloroform procedure. The two mutations T54I and R183W was determined using PCR-RFLP procedure.

**Results:** The AF1III restriction enzyme digested the related restriction site in all of the 100 samples examined. Also, Ss1I restriction site were digested in all of the 100 samples. These data indicate that no T54I and R183W mutations were not detected in deaf individuals tested.

**Conclusion:** Based on data from the present study and previous study, we conclude that DFNB59 gene mutations have a very low contribution to deafness in patients in a province of Iran. However, we examined only 2 DFNB59 mutations in 100 patients and to determine the role of this gene in developing deafness the entire coding region and promoter of the gene need to be investigated in more samples.

#### P10.18

#### DNA repair gene polymorphisms in dialysis patients

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Patients with end-stage renal failure, whether on conservative or haemodialysis therapy, have a high incidence of DNA damage, therefore the potential involvement of DNA damage and its repair are of particular interest. Polymorphisms of DNA repair enzymes may affect repair efficiency and modulate cancer susceptibility. In this study, we aimed to determine the frequency of four polymorphisms in two DNA repair enzyme genes, xeroderma pigmentosum complementation group D (XPD) and X-ray cross-complementing group 1 (XRCC1), in 65 patients undergoing hemodialysis, and 61 subjected to peritoneal dialysis, matched for gender and age with 60 controls. We used polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP), to analyze XPD Asp312Asn, XPD Lys751Gln, XRCC1 Arg194Trp, and XRCC1 Arg399Gln polymorphisms. The genotype distributions in the patients and controls were in Hardy-Weinberg equilibrium for each polymorphism. For each polymorphism there was no significant difference in the genotype distribution between the control group and the dialysis patients, or between the HD and the PD patients (p>0.05). Allele frequencies were also not statistically different between the groups (p>0.05). We conclude that DNA repair gene polymorphism is not associated with renal failure.

#### P10.19

#### Analysis of dopamine D2 receptor (DRD2) gene polymorphisms in cannabinoid users

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Cannabis has been shown to increase the release of dopamine from the nucleus accumbens. Dopamine receptor genes which are expressed in this brain region can be candidates for cannabis addiction. We therefore investigated whether polymorphisms in *DRD2* gene are associated with cannabinoid users in Turkish population.

In this study total 80 cases with cannabinoid users and 100 age-and sex-matched healthy controls were tested for two SNPs which were Taq1-A and Taq1-B in *DRD2* gene. SNPs were genotyped by PCR-RFLP.

The distribution of A1/A1, A1/A2, and A2/A2 genotypes for Taq1-A polymorphism was 11%, 26% and 63% in cases compared with 4%, 25% and 71% in controls ( $p=0.152$ ). The allele frequency of A1 and A2 was 0.242, 0.758 in cases compared with 0.165, 0.835 in controls ( $p=0.09$ ). Also, the distribution of B1/B1, B1/B2 and B2/B2 genotypes for Taq1-B polymorphism was 7%, 24% and 69% in cases compared with 4%, 23% and 73% in controls ( $p=0.576$ ). The allele frequency of B1 and B2 was 0.194, 0.806 in cases compared with 0.155, 0.845 in controls ( $p=0.333$ ).

We conclude that distribution of the genotypes and the allele frequencies were not significantly different between cannabinoid users and controls in *DRD2* gene. However the observed genotype counts was deviated significantly from those expected according to the Hardy-Weinberg Equilibrium (HWE  $p=0.02$  for Taq1-A,  $p=0.03$  for Taq1-B). This is the study that we give the preliminary data is still going on. Further studies with larger samples are needed to address the exact role of *DRD2* gene in cannabinoid users.

#### P10.20

##### Polymorphisms of cytokine genes are associated with endometriosis

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Endometriosis results from the outgrowth of endometrium tissue outside the uterine cavity with subsequent proliferation of the cell and growth in the pelvis. Typical endometrioid cells are characterized by increased cytokine activity. Cytokine genes are polymorphic that results in synthesis of proteins with various functional activities. Our study focuses on the role of allelic variants of IL4, IL4RA, TNF $\alpha$  and RANTES genes in pathogenesis of endometriosis.

DNA samples from the patients with endometriosis (n=120) and healthy women without gynecologic complications (n=91) were included in the study. Polymorphisms of IL4 (-590T>C), IL4RA (1902A>G), TNF $\alpha$  (-238G>A; -308G>A) and RANTES (-403G>A) were defined by PCR-RFLP assay. Levels of TNF $\alpha$  and RANTES in peritoneal fluid were measured by immunochemical analysis.

The alleles and genotypes frequencies of IL4 gene did not differ between the studied groups. The frequency of G/G genotype for IL4RA gene, -308 A/- and -238 A/- genotypes for TNF $\alpha$  gene and -403A/G genotype for RANTES gene were significantly higher in endometriosis patients (18.8%, 42.2%, 44.9%, 48.3%) as compared to the controls ( 1.4%, 8.9%, 9.9%, 27.7%,  $p<0.01$ ). Also the increased level of TNF $\alpha$  and RANTES was found in peritoneal fluid in patients with endometriosis. The presence of rare alleles of IL4RA (1902A>G), TNF $\alpha$  (-308G>A) and RANTES (-403G>A) genes, associated with increased level of corresponding proteins could be treated as a risk factor in development of endometriosis.

#### P10.21

##### Endothelial nitric oxide synthase (eNOS) polymorphisms and haplotypes in Amerindians from Brazilian Amazon

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Disparities in the frequency of endothelial nitric oxide synthase (eNOS) polymorphisms may explain differences in nitric oxide (NO)-mediated effects and response to drugs among black and white subjects. While these differences in the distribution of eNOS polymorphisms have clearly been shown in the American and in the Brazilian populations, there is no information regarding the distribution of eNOS gene alleles

and haplotypes in Amerindians. Hence, we examined the distribution of three clinically relevant eNOS polymorphisms (T<sup>-786</sup>C in the promoter, a variable number of tandem repeats-VNTR- in intron 4, and the Glu298Asp in exon 7) in 170 Amerindians from three tribes of Brazilian Amazon, which are characterized by low admixture levels (2-3%) with nonindigenous people. We also estimated the haplotype frequency and evaluated associations between these alleles, and compared these findings with previously reported results from black and white Brazilians. The Asp298, C<sup>-786</sup> and 4a alleles were less common in Amerindians (5.0%, 3.2% and 4.1%, respectively) than in blacks (15.1%, 19.5% and 32.0%, respectively) and whites (32.8%, 41.9% and 17.9%, respectively) ( $P<0.001$ ). The most common predicted haplotype in the three groups combined only the most common alleles, but exhibited the highest estimated frequency in Amerindians (89%). Our findings are consistent with a lower genetic diversity in Amerindians compared with blacks and whites. These striking interpopulation differences among Amerindian, black and white Brazilians may be of major relevance for case-control association studies focusing on eNOS gene polymorphisms in admixed populations, such as the American and the Brazilian populations.

#### P10.22

##### Presence of 4a allele of 4a/4b eNOS polymorphism may contribute to the risk of complications in post-acute period of myocardial infarction (MI)

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**BACKGROUND:** Endothelial nitric oxide synthase (eNOS) is considered an important enzyme in regulation of vascular tone and therefore may be involved in development of MI. A role of 4a/4b eNOS polymorphism in the risk of MI remains unclear.

**OBJECTIVE:** To investigate genotype distribution and allele frequencies of 4a/4b eNOS polymorphism in male patients of different age with acute myocardial infarction (AMI) and those who had survived MI being at the same age a few years ago.

**STUDY DESIGN:** 13 male patients with AMI under the age of 45 (group I), 57 male patients with AMI older 60 (group II), 275 men who had survived MI being under 45 (group III), and 100 men who had survived MI being older 60 (group IV) a few years ago. 4a/4b eNOS polymorphism was determined by PCR [Taniwaki et al., 2001].

**RESULTS:** Frequency of 4a allele was significantly higher in men from the group I compared to the group III ( $p = 0.047$ , OR = 2.5). The number of 4a allele carriers (men with 4a/4a and 4a/4b genotypes) was twofold in the group I compared to the group III (OR = 2.0 95 % CI 1.17-3.4). There was no difference in the allele frequencies and genotype distribution in men from groups II and IV.

**CONCLUSION:** Our results allow suggesting that presence of 4a allele of 4a/4b eNOS polymorphism may contribute to the risk of complications in post-acute period of MI in young male patients.

#### P10.23

##### The LIPOchip experience in Spain

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DNA-based mutation screening methods usually make the definitive diagnosis of Familial Hypercholesterolemia (FH) and the detection rates vary considerably from 25 to 80%, depending on the clinical criteria used for the diagnosis, the genetic heterogeneity of the population studied and the different analytical methods performed.

To improve the FH genetic diagnosis, we have developed a new diagnostic tool based on a DNA-array system (LIPOchip) which has been used since 2004 by the Spanish Health System. The LIPOchip platform comprises two steps: firstly the genetic analysis of FH patients is achieved by the use of a chip containing the 247 most frequent Spanish mutations (238 LDLR, 3 APOB and 6 PCSK9 mutations). Since around ten percent of LDLR mutations are deletions or duplications of part of the gene, the chip is also able to detect Copy Number Change (CNC) in the LDLR gene. If no mutation is detected by the chip, se-

quencing of the entire LDLR gene is finally carried out. 2462 Spanish index cases were analyzed and mutations were found in 47% of the patients (94% of point mutations and 6% of CNCs). Sequencing has been performed on negative samples and detected 66 new mutations leading to a final percentage of mutation detection of 49%.

We here show that the chip is able to detect nearly 95% of Spanish mutations and allows high throughput genetic diagnosis. We finally show a poor relation between clinical diagnosis (based on Dutch MedPed criteria) and genetic diagnosis, supporting that both diagnosis should be employed.

#### P10.24

#### The spectrum and frequency of MEFV mutations in newborns from Tbilisi, Georgia

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Familial Mediterranean Fever (FMF) is a hereditary inflammatory disorder caused by mutations in the MEFV gene. Carrier rates are known to be particularly high among Sephardic Jews, Turks, Armenians and Arab populations. The spectrum and frequency of MEFV mutations in Georgia has not at all been investigated so far.

Multiplex PCR and reverse-hybridization teststrips (FMF StripAssay) were applied to simultaneously analyze twelve common MEFV mutations in DNA samples from dried blood on filter cards, which had been obtained from 202 unselected newborns at various hospitals in Tbilisi, Georgia. We found 30 samples to be heterozygous and 1 to be compound heterozygous or complex (two mutations in cis). The carrier rate of MEFV mutations (15.3%) was remarkable, although lower than data reported from neighbouring Turkey and Armenia (approx. 20%). The most frequently observed variants were E148Q (15x), M680I G/C (5x) and M694V (4x). Five other MEFV mutations were found at lower incidence (V726A, A744S, R761H: 2x each; P369S, F479L: 1x each). Our data indicate that MEFV mutations, including severe ones such as M680I and M694V, are not uncommon in the Georgian population. Based on these new findings, the awareness for FMF and the availability of appropriate testing should be further promoted in Georgia. (oberkanins@viennalab.co.at)

#### P10.25

#### Fryns syndrome: epidemiological data from 33 European birth registries

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Fryns syndrome (OMIM 229850) is a rare autosomal recessive malformation syndrome. The main features include diaphragmatic hernia, characteristic dysmorphic features, and distal limb anomalies. Additional malformations of central nervous system, gastrointestinal and genitourinary system can be present as well. Because of the rarity and observed phenotypic variability there is a need for better delineation of epidemiological and clinical aspects of this condition. We present data on 22 cases of Fryns syndrome reported to a large European network of congenital malformation registries (EUROCAT) in the 1980-2002 period. Prenatal ultrasound examination detected abnormalities in 13/22 (59%) fetuses. Mean gestational age at discovery of an abnormality by prenatal ultrasound was 22±3.9 (18-33) gestational weeks. Congenital diaphragmatic hernia (20/22 or 91%), limb defects (16/22 or 72.7%), genitourinary tract anomalies (16/22 or 72.7%) and cleft palate (10/22 or 45.5%) were the most frequently found malformations. There were 4/24 (18.2%) fetal deaths, 12/22 (54.54%) pregnancy terminations and only 6/22 (27.3%) live born. Male: female ratio was 2 (14/7). The mean gestational age at birth was 33 weeks. The mean live birth weight was 1591±967g for males and 2075±125g for females. Only one newborn survived the first week of life. Parental consanguinity was present in 11/22 (50%) instances. In 9/22 (41%) cases previous siblings with anomalies were noted, but in only one case Fryns syndrome was con-

firmed. Karyotyping was performed in 9 cases and no chromosomal abnormality was found. No evidence of specific teratogenic exposure was observed.

#### P10.26

#### Molecular Identification of the most prevalent mutations of glucose-6-phosphat dehydrogenase(G6PD) gene isolated in two provinces, Fars and Esfahan of Iran

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human enzyme defect, being present in more than 400 million people worldwide. It catalyses the oxidation of glucose-6-phosphat to 6-phospho Gluconat in the first committed step of the pentose phosphate pathway, which provides cells with pentoses and reducing power in the form of NADPH. fauvism disease causes hemolytic anemia impressible of fava bean and primaquine anti-malaria drug. Genetic defect caused by mutations in the G6PD gene, resulting in variants with wide range of biochemical and clinical phenotypes. Recently have been shown that prevalence of G6PD deficiency in regions with malaria provides circumstantial evidence that G6PD deficiency confers resistance against malaria.

The aim of this study was the molecular analysis of common G6PD mutations (Mediterranean, Chatham, Cosenza and A-(G202A/A367G) in the patient with fauvism.

Studies on G6PD deficiency in Fars and Esfahan provinces were performed in 92 patients with a history of fauvism which collected 34 and 62 samples, respectively. genomic DNA with PCR-RFLP method analyzed for known mutations such as; Mediterranean(C-T)nt, Chatham, Cozensa and A202(G-A)/367(A-G) mutation ( PCR-RFLP).

Our results indicated that, two different major polymorphic variants were found: from the total 96 samples, 79 had G6PD Mediterranean (82.3%) and 8 sample had G6PD Chatham (8.33%), and none of the samples had Cozensa, A-(G202A/A367G) mutation AND remained unknown.

Conclusions: Result showed that G6PD Mediterranean was the most prevalent mutation in this two province which is similar to results of other studied provinces in Iran.

#### P10.27

#### Study of genes for glutathione S-transferases GSTT1 and GSTM1 in patients with liver cirrhosis in Siberian part of Russia

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Glutathione S-transferases (GSTs) are phase II xenobiotic metabolizing enzymes which intended for the detoxification of electrophilic compounds, including, therapeutic drugs, environmental carcinogens, acetaldehyde and products of oxidative stress. Oxidative stress is important pathogenic factor in liver damage related to alcohol and hepatitis B and C.

We investigated association for homozygous deletion (null genotype) of genes GSTT1 and GSTM1 with a cirrhosis of a liver and its etiology. The group of patients with a cirrhosis consisted of 189 inhabitants of Tomsk area of Russia. Among them were patients with alcoholic cirrhosis (n=82), viral induced cirrhosis (n=34) and cirrhosis of mixed etiology (n=73). The 135 inhabitants of Tomsk was population control group.

Comparison of null alleles in the group with liver cirrhosis with control group has shown decreased frequency of null genotype for GSTM1 (39.2% and 54.8% in cases and controls, respectively, p=0.007) and null genotypes for GSTT1 and GSTM1 (7.9% and 15.6%, p=0.048).

There were no differences in frequency of null genotypes for genes GSTT1 and GSTM1 between subgroups of patients with a cirrhosis of various etiology. However, patients with alcoholic cirrhosis and cirrhosis of mixed etiology were characterized by lower frequency of null genotype for gene GSTM1 comparing to control group (39%, 34.2% and 54.8%, p=0.034; p=0.007). We suppose, that null genotype gene

*GSTM1* could be protective concerning alcoholic cirrhosis and cirrhosis of mixed etiology.

#### P10.28

#### Distribution of polymorphisms in genes for interleukins and their receptors in different ethnic groups of Siberia

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Distribution of polymorphisms in genes *IL4* (rs2243291), *IL4RA* (rs1801275 and rs2074570), *IL12A* (rs568408), *IL12B* (rs3212227 and rs3212220) and *IL12RB1* (rs3746190 and rs11575926) in four ethnic groups of Siberian region (the immigrant Caucasian population - Russians, and indigenous Mongoloid populations - Yakuts, Tuvians, Buryats; 96 individuals in each group) was studied.

Comparison of allelic frequencies with the data on other populations worldwide [http://www.ncbi.nlm.nih.gov] has shown that for six out of eight investigated SNPs the frequencies were beyond the values known for Caucasians and Mongoloids (except for *IL4RA* (rs1801275) and *IL12B* (rs3212220)). Allelic frequencies for SNPs in *IL4* (rs2243291), *IL4RA* (rs2074570), *IL12RB1* (rs11575926), *IL12B* (rs3212220) are significantly different between Russian and indigenous populations of Siberia. For two SNPs (rs3746190 in gene *IL12RB1* and rs3212227 in gene *IL12B*) ethnic differences in distribution of allelic frequencies were shown. No ethnic specificity was shown for distribution of allelic frequencies for rs568408 in *IL12A* and rs1801275 in *IL4RA*. According to pairwise  $F_{ST}$  values calculated for studied SNPs in four ethnic groups, the extent of genetic differentiation in Siberia makes up 3.71 %. We observed that only Buryats and Yakuts do not show statistically significant distinctions in their genetic structure.

The results once again testify high genetic heterogeneity of ethnic groups in Siberian region which has to be considered while planning genetic-epidemiological studies of common diseases.

#### P10.29

#### X-chromosomal haplotypes in global human populations

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To reconstruct the origin and evolution of X-chromosomal lineages in global human populations we investigated the genetic diversity in 23 population samples (about 1500 individuals totally) using SNP markers in a single linkage disequilibrium region of *ZFX* gene. About sixty haplotypes belonging to 3 phylogenetic branches (A, B, and F) originated from the single African root were found in the total sample. Branch A includes mostly African haplotypes, whereas four major haplotypes belonging to different sub-branches of B (haplotype E8) and F (haplotypes H4, I3 and I11) were present in Eurasia. Major haplotype of the older branch B (E8) is almost evenly distributed among Eurasian populations. Haplotypes of the younger phylogenetic branches demonstrates clinal distribution with the sharp frequency changes from East to West. Haplotype H4 is presumably "Eastern-Eurasian". It reaches the highest frequency in Eastern and South-Eastern Asians. Haplotypes I3 and I11 in the contrary show the clear frequency gradient from West to East with the highest frequency in Europeans, moderate frequency in Central Asia, and the minimal frequency in North-East and South-East Asia. The total level of genetic differentiation of global human populations estimated by the analysis of molecular variance of X-chromosomal haplotypes ( $F_{ST} = 9.1\%$ ) is quite high and roughly corresponds to those measured for most other types of genetic markers except Y-chromosomal haplogroups which are characterized by the much higher level of between-population differences.

#### P10.30

#### Genetic Position of Bucharest region (Romania) in the According to Eight DNA markers

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Historically, the Bucharest city that was founded at the end of XIV century played a key role as a trade route for south-eastern civilization, and it has been the setting for numerous conquests and demographic expansions.

In this work, eight DNA markers (rs4646994, rs61722009, rs7975232,

rs731236, rs2228570, rs1544410, rs1801133, rs1805087) were typed in 100 unrelated individuals (sex ratio 60:40) from Bucharest region (Romania). Aim was to analyze the genetic variability and to establish the relation between this region and other European populations.

Allele frequencies were calculated by direct counting; Hardy-Weinberg equilibrium was assessed by an exact test. Gene diversity for each population was calculated. Genetic distances matrices were represented using Nei's method by PHYLIP 3.68 package. Tree topology was inferred using the Neighbor-Joining method and assessed through 1,000 bootstrap iterations.

In our lot, the most common polymorphism was MTR-rs1805087 (82.5%) and the least frequent was VDR- rs731236 (50%). The gene diversity compute was 0.545. Polymorphism's frequencies were similar to the mean frequencies calculated for the whole set of populations included in the study. The most affiliated population with our lot are Italy, Spain, Poland, Germany, Greece and Turkey the most distant population are United Kingdom, Sweden, Croatia and Slovakia.

This study indicates that data on SNPs provide information about the evolutionary history of human populations. In the future other genetic markers will be studied to be compared with the results obtained in neighboring populations to elucidate more precisely the genetic structure of this region of the Balkanic Peninsula.

#### P10.31

#### Genetic differences between four European populations

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Population stratification can distort the results of genome-wide association studies (GWAS). One approach to deal with this inflation of the statistic is to estimate the inflation factor and adjust the detection statistic accordingly. However, the evolutionarily forces work with different strength in some regions of the human genome, e.g. around the lactase gene (LCT) and the HLA region, making such an adjustment inappropriate.

We examined the population differences in four European populations (Scotland, Ireland, Sweden and Bulgaria) using data from GWAS performed with the Affymetrix 6.0 array at the Broad Institute. We show that there are >20,000 SNPs which are highly ( $p < 10^{-6}$ ) significantly stratified between the four populations, after genome wide Bonferroni correction for multiple testing. We then examined the top 20 stratified regions to see what genes might have caused the top differences, using a highly conservative cut-off of  $p < 10^{-40}$ . Some of the loci span genes reported before: hair colour and pigmentation (HERC2, EXOC2), the LCT gene, genes involved in NAD metabolism, and genes involved in immunity (HLA and the Toll-like receptor genes TLR10, TLR 1, TLR 6). Among the top hits were several genes which have not yet been reported as stratified within European populations, indicating that they might also provide a selective advantage. Some involve other immunity genes (CD99, ILT6), but others show no obvious effect on positive selection: several zinc fingers, and most intriguingly, FOXP2, implicated in speech development. Future GWAS should take into consideration any positive associations with these genes.

#### P10.32

#### Conditional linkage and genome-wide association studies identify UGT1A1 as major gene for anti-atherogenic serum bilirubin levels - a Framingham Heart Study

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Objective and Methods: Low bilirubin levels are significantly associated with cardiovascular diseases (CVD). In previous genome-wide linkage studies we identified a major locus on chromosome 2q harboring the

candidate gene UDP-glucuronosyltransferase (UGT1A1). The activity of this enzyme is significantly influenced by a TA-repeat polymorphism in the promoter of the gene. In a prospective study individuals with genotype (TA)7/(TA)7 had significantly higher bilirubin levels and approximately one third the risk of CVD as carriers of the wild type (TA)6 allele. In the present study we performed a conditional linkage study to investigate whether this polymorphism explains the observed linkage peak and extended our analysis by a genome-wide association study on bilirubin levels in 1345 individuals.

Results: After adjustment for the bilirubin variance explained by this polymorphism, the LOD score on chromosome 2q dropped from 3.8 to 0.4, demonstrating that this polymorphism explains the previous linkage result. For the genome-wide association study, the closest marker to UGT1A1 was in the top ranking SNPs. The association became even stronger when we considered the TA-repeat polymorphism in the analysis ( $p=2.68 \times 10^{-53}$ ). Five other SNPs in other regions reached genome-wide significance without obvious connection to bilirubin metabolism.

Conclusions: Our studies suggest that UGT1A1 may be the major gene with strong effects on bilirubin levels and the TA-repeat polymorphism might be the key polymorphism within the gene controlling bilirubin levels. Since this polymorphism has a high frequency and a substantial impact on the development of CVD, the gene might be an important drug target.

#### P10.33

#### Polymorphism of some cytokines genes in connection with age gradation

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Aim of study was to investigate *IL6* (-572G/C), *IL10* (-627C/A), *IL12B* (1159A/C) and *TNFA* (-308G/A) gene polymorphisms in connection with age gradation.

Total group (1627 individuals, from 1 to 109 years old, ethnic Tatars, Russia) was divided into young (1-20), middle-age (21-55), aged (56-74), senile (75-89) and long-living (90-109) persons. Gene polymorphism was analyzed by PCR-RFLP. Fisher's two-tailed exact test was used for age groups comparison.

Among aged persons *IL6*(-527)\*G/\*G genotype frequency was higher than in middle-age group (80.0% vs. 69.28%,  $P=0.004$ ). *IL6*(-527)\*G/\*C genotype frequency was lower in group of long-livers than in middle-age group (15.79% vs. 27.9%,  $P=0.006$ ). *IL6*(-527)\*C/\*C genotype frequency was lower in aged (0%,  $P=0.005$ ) and senile groups (0.56%,  $P=0.03$ ) than in middle-age group (2.82%). In middle-age group *IL10*(-627)\*C/\*C genotype frequency (56.09%) was higher in comparison with aged (47.83%,  $P=0.034$ ), senile (43.53%,  $P=0.005$ ) and long-livers groups (43.28%,  $P=0.015$ ). Accordingly, in this group *IL10*(-627)\*C/\*A genotype frequency (34.56%) was lower than in aged (42.61%,  $P=0.03$ ), senile (45.54%,  $P=0.002$ ) and long-livers groups (46.27%,  $P=0.021$ ). *IL12B*(1159A/C)\*C/\*C genotype frequency is higher in senile group than in middle-age group (6.78% vs. 3.21%,  $P=0.049$ ). In aged and senile groups *TNFA*(-308)\*G/\*G genotype frequencies were 71.39% and 79.37%, *TNFA*(-308)\*A/\*G genotype frequencies - 26.08% and 18.53% accordingly ( $P=0.02$ ).

Thus, genotypes frequencies changes of studied genes in various age groups are traced. This data allow considering cytokines genes as associated with life span.

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#### P10.34

#### Genetic study of 45 big hearing loss pedigree and *GJB2* gene mutations frequency in a province of Iran

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Republic of Iran.

Background and aim: Hearing loss is the most common sensorineural disorder in human. This disorder is heterogeneous and happens due to genetic or environmental causes or both. Mutations in the *GJB2* gene have been involved in deafness in many populations. This study aims to investigate genetic epidemiology and frequency of *GJB2* gene mutations in 45 big deaf pedigrees.

Methods: In this genetic epidemiology study we have investigated 45 big deaf pedigrees concerning inheritance patterns consanguinity and diversity of deafness severity among siblings using data collected by questionnaires and audiograms. We examined also the frequency and profile of *GJB2* gene mutations in 45 probands using direct sequencing strategy.

Results: Our study revealed 73% of consanguinity in the deaf pedigrees studied from which first cousins marriage was the more common with the rate of 49%. The most common type of first cousins marriage was found between first cousin who were the children of two brothers. We found autosomal recessive and X-linked recessive pattern in 94-97% and 3-6% of the pedigrees studied respectively.

We found also 57% of diversity (mild to profound) of deafness severity among deaf siblings *GJB2* mutations were found in 11% of population studied including 35delG, 167delT, 299-300delAT and 363delC.

Conclusion: A high rate of consanguineous marriage determined in this study could rise the rate of autosomal recessive patterns up to 94-97% of the overall pedigree and would be the main cause of congenital deafness. This study revealed a low contribution of *GJB2* gene mutations in Chaharmahal va Bakhtiari Province.

#### P10.35

#### *GJB2* Carrier screening with ultrasonographic measurement of epidermal thickness

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Inherited hearing loss (IHL), affecting 1/1000 children, represents an important public health issue and implies disability, expensive interventions and rehabilitation. The most frequent cause of IHL, due to mutations in *GJB2* gene, presents a high carrier frequency worldwide. There are no means to identify them apart from expensive DNA-methods. Recently high epidermal thickening due to *GJB2* carrier status was suggested. We used skin ultrasonography measurement of epidermal thickness in a series of controls and obligate carriers. 273 individuals were tested by ultrasonography using a linear band probe to determine their epidermal thickness. Variance and linear regression analyses were carried out. Regression coefficients were used to obtain scores of thickness corrected by age and sex. Carriers presented significant increase in epidermal thickness as compared to controls, *GJB2* status explaining 50% of this variability. Results led to the development of a screening protocol with 98% of sensitivity and 93% specificity, in subjects aged 20-80, with a likelihood ratio of a positive test of 14 to 1. Better results were obtained studying people in reproductive age. The availability of a simple, non-invasive, rapid, sensitive, and cheap ultrasonographic protocol opens new perspectives for carrier screening in the population. Present data further supports the role of skin thickness as major selective advantage for *GJB2* carriers.

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#### P10.36

#### Genome-wide Interaction Analysis Guided by A Priori Information

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Complex diseases are caused by interacting genetic and environmental factors. Due to computational burden, genome-wide association studies (GWAS) are typically limited to single-marker analysis. We present

an approach for genome-wide interaction analysis that overcomes the computational issue by prioritizing SNPs for interaction analysis using a priori information. Sources of information can be biological relevance (common pathway, gene location ...) or statistical evidence (single marker association at a moderate level). We present a respective software product that implements different approaches to joint analysis of multiple SNPs (full modelling of marginal and interaction effects as well as explicit testing for association with a log-linear model). Moreover, link to databases with the relevant biological information is provided. We also present the results from an application to a GWAS.

#### P10.37

##### Heredity Nervous System Diseases in Rostov Region

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Research of Hereditary Nervous System Diseases is important for the following reasons: their prevalence among neurological disorders is high. Many patients develop mental and physical handicap leading to severe disability. The population of eight districts was examined to access prevalence of hereditary nervous system disorders in Rostov Region. Data collection and processing were done according to the protocol of Genetic Epidemiology Laboratory of Medical Centre in Moscow. The neuromuscular disorders in Rostov Region's population composes 30.62% of all hereditary nervous system diseases. Their prevalence is 19.94 per 100,000 population. Hereditary Sensory-Motor Neuropathy was prevailing not only in the group of neuromuscular diseases, but also in hereditary nervous system disorders group. Total of 28 cases were identified in 15 families. The prevalence was 8.72 per 100,000 population. DNA testing was performed to identify dup mutation in gene RMR22 in all patients.

This study identified 13 cases of Duchene/Becker Muscular Dystrophy in 10 families. Prevalence was 8,10 per 100,000 male population. DNA testing was performed to identify mutations in gene DMD in 9 families.

Other common disorders such as oligophrenia, microcephaly and congenital hydrocephalus comprise 47.6 % of all hereditary nervous system diseases in Rostov Region; prevalence was 30,85 per 100,000 population.

Heredity Nervous System Diseases, Duchenne/Becker Muscular Dystrophy, Neurofibromatosis and Tuberous Sclerosis are prevailing disorders among Rostov Region's population. All this diseases are single gene disorders, which is also true for Russian Federation neuropathology spectrum.

#### P10.38

##### Confounding factors in testing parent-of-origin effects: illustration with Hirschsprung disease genetics.

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Parent-of-origin effect may be tested on affected sib pairs or trio family data by comparing paternal and maternal transmission to the affected cases. However, the conclusion of these tests is not robust to non random parental mating. In particular, we demonstrate, through theoretical modelling, that reduced fertility and spermatic fitness can mimic a parent-of-origin effect both for sib pairs and case-parents trios. In order to distinguish between true parent-of-origin effect and bias resulting from reduced fertility, we propose here a method which combines the information on trios and sib-pairs.

We illustrate our findings with the International Hirschsprung disease (HSCR) Consortium data. *RET* is the major HSCR locus and a parent-of-origin effect has been suspected. Both the strong sex-ratio in favour of females combined with a poor prognosis and the involvement of *RET* in spermatogenesis have led to challenge a parent-of-origin effect. Indeed, by combining the results of affected sib-pairs and trios, we show that a reduced fertility is the most likely mechanism to explain the observed allele sharing distortion in HSCR.

#### P10.39

##### Worldwide HLA Class I and II Diversity

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The Human Leukocyte Antigen (HLA) is a key component of the immune system of vertebrate as it is responsible for the recognition and presentation of antigens and comprises the most polymorphic genes in vertebrates. Numerous hypotheses have been proposed to explain how this diversity could be maintained. One such hypothesis (called Pathogen-Driven Balancing Selection) states that different alleles provide protection against different pathogens. A prediction under this hypothesis is that populations exposed to a wider variety of diseases should be characterised by higher diversity at their HLA genes. This prediction has recently received some support in humans when it was shown that once past demography was accounted for, there is a positive correlation between HLA class I within-population genetic diversity and the number of endemic diseases found in that area.

My work involves compiling a large database of HLA allele frequencies from human populations worldwide, including class I and II genes. One limitation of previous work was that a very simple measure of genetic diversity was computed, and the different alleles were simply considered as identical or different. Thus, my work involves using more refined measures of genetic diversity and focuses on the residues forming the Peptide Binding Region (PBR), as these are the expected target of natural selection.

The work indicates that the geographic apportionment of HLA diversity is the product of both past demography and complex selective pressures. It also highlights the complexity of the selective process, with the likely involvement of coevolution with KIR genes and marks differences between different classes of genes.

#### P10.40

##### HLA polymorphisms in São Tomé and Príncipe Archipelago (West-Africa)

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HLA-A, HLA-B, and HLA-DRB1 loci polymorphisms were high-resolution typed through sequence based typing in *Forros* and *Angolares* ethnic groups from São Tomé and Príncipe archipelago (West-Africa). The most frequent HLA-A alleles found in *Forros* were A\*0201, A\*2301 and A\*6802, with 13% each. With a similar frequency (14%), HLA-A\*6802 was also a very common allele in *Angolares*, followed by A\*2301 (9.2%). HLA-B\*5301 was the most frequent HLA-B allele in both *Forros* (19%) and *Angolares* (24%). The most recurrent HLA-DRB1 alleles in São Tomé and Príncipe population were HLA-DRB1\*0301 (12% in *Forros* and 18% in *Angolares*) and HLA-DRB1\*1503 (11% in both *Forros* and *Angolares*). Haplotypes A\*6802-B\*0702-DRB1\*1301 in *Forros* (3%) and A\*6802-B\*5301-DRB1\*0804 in *Angolares* (4.7%) were the most common three loci found in each group.

Phylogenetic analysis reveals the West Coast of Africa as being the place of origin of São Tomé and Príncipe main genetic pool, specifically the area comprised between Guinea-Bissau and the Gulf of Guinea. *Forros* and *Angolares* systematically cluster together in phylogenetic analysis and are not statistically different, making the hypothesis that *Angolares* are descendants of slaves who escaped from plantations and kept themselves resistant to miscegenation plausible.

#### P10.41

##### Genomic runs of homozygosity: population history and disease

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Runs of homozygosity (ROH), resulting from the inheritance from both parents of identical haplotypes, are abundant in the human genome. ROH length is determined partly by the number of generations since the common ancestor: offspring of cousin matings have long ROH, while the numerous shorter ROH reflect shared ancestry tens and hundreds of generations ago. In studies of European populations we show that  $F_{roh}$ , a multipoint estimate of individual autozygosity derived from genomic ROH, distinguishes clearly between subpopulations classified in terms of demographic history and correlates strongly with pedigree-derived inbreeding coefficients. In a global population da-

taset, analysis of ROH allows categorisation of individuals into four major groups, inferred to have (a) parental relatedness in the last 150 years (many south and west Asians), (b) shared parental ancestry arising hundreds to thousands of years ago through population isolation and restricted effective population size ( $N_e$ ), but little recent inbreeding (Oceanians, African hunter-gatherers, some European and south Asian isolates), (c) both ancient and recent parental relatedness (Native Americans), and (d) only the background level of shared ancestry relating to continental  $N_e$  (east Asians, urban Europeans; African agriculturalists). Long runs of homozygosity are therefore a widespread and underappreciated characteristic of our genomes which record past consanguinity and population isolation and provide a unique record of individual demographic history. Individual ROH measures also allow quantification of the disease risk arising from polygenic recessive effects. We present preliminary data from a survey of the effects of ROH on quantitative disease-related traits and disease risk.

#### P10.42

##### Detection and quantitation of mu opioid receptor splice variants mRNA in peripheral blood lymphocytes of opioid addicts

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**Aims:** In the present study we have investigated the presence and changes of PBLs mRNA expression of four human mu opioid receptor splice variants (hMOR-1A, hMOR-1O, hMOR-1X, and hMOR-1Y) in the opioid addiction process to see whether they can serve as peripheral markers.

**Design:** Splice variants mRNA expression in PBLs was detected and measured by real-time PCR using SYBR green dye.

**Participants:** Four groups, each comprising of 30 male individuals were included: opioid addicts, methadone maintained patients, long-term abstinent former opioid addicts, and non-addicted control subjects.

**Findings:** hMOR-1A and hMOR-1O are expressed in PBLs. However, we did not observe PBLs expression of hMOR-1X and hMOR-1Y. The hMOR-1A expression was reduced in abstinent (reaching 0.33 the amount of control group), up-regulated by the factor 1.94 in methadone maintained patients and not statistically different in addicted group in comparison to controls. The hMOR-1O expression was significantly reduced in abstinent and methadone maintained subjects reaching 0.39 and 0.53 the amount of control group, respectively. However, addicted group was not statistically different from controls.

**Conclusions:** Deficiency in expression of hMOR-1A and hMOR-1O splice variants measured by a suggested peripheral marker, may be a risk factor making individuals susceptible for drug addiction. This deficiency reaches to nearly normal levels in opioid addicts. However, from therapeutic point of view, we have no explanation for hMOR-1A mRNA up-regulation and hMOR-1O mRNA down-regulation in methadone maintained subjects at the moment.

**Keywords:** human, lymphocytes, mRNA expression, mu opioid receptor splice variants, opioids

#### P10.43

##### Coevolution of the repeated glutamine and proline codons in the mammalian Huntington disease gene

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The human Huntington disease (*HD*) gene contains a CAG microsatellite encoding a polyglutamine tract, followed by cryptically simple regions encoding a proline reach region. The *HD* gene is evolutionary conserved between *Drosophila* and humans, but repeated CAG codons are seen only in the vertebrates, while repeated CGG codons are present only in the mammals. To improve understanding of the evolution of the *HD* gene repeated codons, we sequenced that part of the gene in 17 mammalian species. The analysis of the obtained nucleotide and supposed protein sequences, as well as database sequences of non-mammalian species, led to a model that predicts the coevolution of repeated glutamine and proline codons in the mammalian *HD* gene. The coevolution probably resulted from interplay between mu-

tational processes, such as replication slippage and point mutations, and selection, such as purifying selection and selection on the reading frames in which tandemly repeated codons can accumulate. The balance between these processes significantly differ in two regions: replication slippage and strong purifying selection probably were the main force for the evolution of the repeated glutamine codons, while synonymous and non-synonymous point mutation and weaker purifying selection drove evolution of repeated proline codons. Analyzed region of the *HD* gene is an example for rapid evolutionary change in an evolutionary ancient gene, leading to creating homopeptide regions, which could assign huntingtin protein with some new functions.

#### P10.44

##### The estimation the age of the founder mutation causing autosomal recessive hypotrichosis in Chuvash and Mari Republics of Russia

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Chuvash and Mari populations of Russia is of great interest, as three endemic autosomal recessive diseases has been recorded with high frequency in these regions: erythrocytosis (OMIM#263400), osteopetrosis (OMIM#259700), and hypotrichosis (OMIM#604379). The presence of such genetic differentiation in the populations presumably results from the long reproductive isolation of the populations and the genetic drift. It was established the founder mutations caused this conditions - Arg200Trp in VHL gene, c.807+5G>A in TCIRG1 gene and ex 4 del in LIPH gene correspondingly. Recently, the age of the mutation for erythrocytosis and osteopetrosis in Chuvashians calculated and was average 1,250 and 890 years.

In this work to estimate the age of the founder mutation for hypotrichosis in Chuvash and Mari populations we were analyze five microsatellite markers (D3S3730, D3S3609, D3S3592, D3S1262 and D3S3600) flanking the LIPH gene in 18 Chuvash and 20 Mari chromosomes bearing the mutation and in populations chromosomes (74 and 86). The mutation was associated with D3S3609, D3S3592 and D3S1262 in Chuvashians and with D3S3609 and D3S3592 in Marians. The mean age was equal to 26.3 generations for Chuvashians and 35.67 generations for Marians, i.e. ~ 720 and 1070 years. These results assume that bottle neck was 700 - 1000 years ago in these regions.

#### P10.45

##### The Linkage Disequilibrium Pattern of *IGF-1* Promoter Polymorphism

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**Introduction:** Insulin-like growth factor-I (*IGF-I*) is a strong risk factor of various cancers including colon cancer, prostate cancer and premenopausal breast cancer. Some studies suggested that the length of a cytosine-adenine (CA)n repeat polymorphism located in the promoter 1 region 970bp upstream of transcription start site (TSS) of *IGF-I* gene is proportional to the *IGF-I* expression, while some others failed to reproduce this relationship. Therefore, we hypothesized that there may be some other genetic variations in the *IGF-I* promoter region contributing to the high activity of *IGF-I* other than the -970 CA microsatellite repeat. The objective of this study was to define the relevant haplotypes of *IGF-I* promoter in the Chinese population.

**Methods and results:** One hundred and sixty male Chinese subjects participated in this study. Three identified SNPs (-603 T/A, -705 T/C, -1410 T/C) and the -970 CA microsatellite repeat in the *IGF-I* promoter 1 were selected and genotyped by restriction fragment length polymorphism (RFLP). The results showed that three haplotypes, C-18-TT, C-19-TT, and T-21-CA, are prevalent, and they account for almost 80% of genotypes in the Chinese population.

**Conclusion:** The haplotype pattern, together with the CA microsatellite repeat number, may be a biomarker for various cancers in the Chinese population. Additional studies are needed to investigate the different transactivation activity of the prevalent haplotypes in the Chinese population.

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#### P10.46

#### Interleukin-23 receptor gene polymorphisms in Hungarian patients with psoriasis

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**Background:** Psoriasis is a chronic inflammatory disease that affects the skin and joints. Its prevalence rates vary from 0.5% to 4.6% between countries and races, affecting 2-3% of whites of European descent. Recently, associations were found between several autoimmune diseases including psoriasis and variants of interleukin-23 receptor (IL23R) gene. In our current work we analysed the association of nine polymorphisms of IL23R with psoriasis in Hungarian samples. **Methods:** Groups of patients with psoriasis (n=214) and unrelated, clinically healthy control subjects (n=192) were genotyped using PCR-RFLP methods. **Results:** We observed a significant increase in the carriage of the minor allele of rs11805303 in the psoriasis group compared to controls conferring a 1.55-fold risk for the development of psoriasis (p=0.029; OR=1.55; 95% CI: 1.05-2.29). Similarly, in psoriasis patients the homozygous carrying of the minor allele of rs2201841 or rs10889677 conferred a more than 2-, and 3-fold increase for the development of psoriasis, respectively (for rs2201841: p=0.024; OR=2.41; 95% CI: 1.12-5.15; for rs10889677: p=0.008; OR=3.04; 95% CI: 1.34-6.92). **Conclusions:** We confirmed the associations of IL23R polymorphisms with psoriasis in a Hungarian population, and were first to demonstrate the effect of the rs11805303 intronic SNP on the disease.

#### P10.47

#### The Incidence of Inborn Errors of Metabolism (IEM) in Eastern Province of Saudi Arabia

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**Objective:** To determine the number of children born with Inborn Errors of Metabolism (IEM) from 1983-2008 within the Saudi Aramco Medical Services Organization (SAMSO) medical centers. This population is a relatively stable well defined population of the Eastern province of Saudi Arabia where a thorough follow up system is provided to all the patients.

**Methods:** The records of all patients, born from January 1983-December 2008 within SAMSO facility and diagnosed with IEM.

**Results:** Over the last 25 years, 165,530 infants were born and followed up at SAMSO. Among these infants, 248 were diagnosed with different types of IEM. The over all estimated incidence was 150 cases per 100,000 live births. 54 % of these were labeled as small molecules disorders such as aminoacidopathy, organic acidopathy, urea cycle disorders and fatty acids oxidation disorders. Organic acidopathy was the most commonly diagnosed small molecule disorder (29/100,000). Lysosomal Storage Diseases (LSD) was diagnosed in 31.5 % of children. 43 % of these were mucopolysaccharidosis (MPS) and the most common type was MPS VI. The incidence per 100,000 live births for each group was estimated.

**Conclusion:** Our data provides a good estimate of IEM incidence in the population studied. Consanguinity plays a major role in the high incidence of single gene autosomal recessive disorders among this population. However, we believe this data still underestimated the true incidence. Therefore, regional newborn screening program will help provide the best estimation of the incidence of IEM in this population.

#### P10.48

#### Is there a need for functionally-enriched tagSNPs sets in candidate gene case-control association studies? An *IRAK3* gene re-sequencing data exploration

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Interleukin-1 receptor-associated kinase 3 gene (*IRAK3*) is a candidate susceptibility gene for complex disease processes such as asthma and sepsis. Before validating these associations in the Spanish population, here we used re-sequencing data of the gene from this population to select different sets of tagging SNPs (tagSNPs), one including potentially functional variants (set1) and a second set unselected for their functionality (set2), and compared their accuracy in predicting untyped variants. Additionally, we explored how well a set of tagSNPs chosen from the European HapMap samples data (set3) performed in the Spanish population. These three sets were used to estimate minor allele frequencies (MAFs) of untyped variants using TUNA software. A high correlation between observed and estimated MAFs was found for all three sets ( $R^2 > 0.95$ ). Differences between observed and estimated MAFs were similar for set1 and set2 (~7%) and were much higher for set3 (34%), being reduced by two-fold (1.7%, 2.7% and 17%, respectively) for common variants ( $MAF \geq 10\%$ ). On the basis of these results, we propose that only common ( $MAF \geq 10\%$ ) untyped variants of this gene should be considered for association studies, especially when tagSNPs are selected from a reference HapMap population. Although functionally-enriched tagSNPs sets might be a straightforward way of exploring the association of a candidate gene with disease, their use increase genotyping costs without improving the accuracy in predicting untyped variants or the coverage of the gene.

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#### P10.49

#### Association of IL-10 promoter genetic polymorphisms with the risk of Kawasaki Disease and outcome of coronary artery lesions

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Kawasaki disease (KD) is the most common cause of paediatric acquired heart disease. Recent studies have indicated that the acute KD patients were with 3-33 fold higher levels of interleukin-10 (IL-10) in plasma. Therefore, a family-based association study of 101 case-parents trios and a matched case-control study of 76 KD cases with coronary artery lesions (CALs) and 76 KD controls without CALs were carried out to evaluate the association of SNPs in IL-10 promoter (-1082, -819, and -592) with the risk of KD and CALs. Based on the TDT results, there were significant differences in the transmission of IL-10-819 and -592 SNPs ( $p=0.005$  and  $0.012$ , respectively). In addition, the TC and CC genotypes of IL-10-819T>C were associated with the increased risk of KD (AOR, 1.33; 95%CI, 0.71-2.50 and AOR, 3.25; 95%CI, 1.15-9.22, respectively) but decreased risk of CALs (AOR, 0.93; 95%CI, 0.47-1.81 and AOR, 0.074; 95%CI, 0.01-0.62, respectively), as compared to TT genotype. The AC and CC genotypes of IL-10-592A>C were with borderline significance of increased risk of KD but decreased risk of CALs (AOR, 0.90; 95%CI, 0.46-1.75 and AOR, 0.03; 95%CI, 0.03-0.87, respectively), as compared to AA genotype. Furthermore, as compared with TA/TA diplotype (defined by -819T>C and -592A>C), CC/CC diplotype of IL10 was associated with the increased risk of KD (AOR, 3.25; 95%CI, 1.15-9.22) but with the decreased risk of CALs (AOR, 0.18; 95%CI, 0.04-0.72). In conclusion, IL10-819 and -592 SNPs played important but contrary roles in the susceptibility of KD and CALs outcome.

#### P10.50

#### Kinship and fertility in a fast-growing population

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The relation between kinship and fertility has been investigated in many populations, with mixed results regarding the advantage or disadvantage of consanguineous unions on reproductive success. In most cases though, measures of kinship were limited to close links due to lack of genealogical data. In this study we investigated a to-

tal of 24728 couples who married between 1840 and 1944 in the Saguenay-Lac-St-Jean (SLSJ) region of Quebec (Canada). Genealogical and fertility data were obtained from the BALSAC population register. Most genealogical branches go back to the early 17<sup>th</sup> century, with an average depth of nearly 10 generations. All genealogical links between spouses of each couple were measured. Kinship coefficients were computed by cumulating kinship links at each generation level, up to the tenth generation. Fertility measures include total number of children, married children and grandchildren for each couple. For most of the period, fertility levels remained high in this population. On average, SLSJ couples had 7 children, 4 married children and 21 grandchildren. Due to high levels of genealogical depth and completeness, at least one kinship link was found for nearly all couples (94%). At the tenth generation, the average kinship coefficient reaches a value of 0.0076 which is almost 5 times higher than the average value for close kinship (< 5 generations). Couples who share at least one ancestor before the seventh generation have on average 7.4 children, compared to 6.9 children for couples with no kinship link.

#### P10.51

##### Studying human genome variation in malaria-endemic populations

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Malaria is an enormous burden to global health care and represents the strongest known evolutionary pressure on human genome. The Malaria Genomic Epidemiology Network Consortium Project 3 (Malaria-GEN CP3) assesses genetic variability in malaria-associated genomic regions within and across malaria-endemic populations by re-sequencing. Regions are prioritised based on their significance in genome-wide association (GWA) studies and by their perceived relevance to the medical research community. Each participating study site aims to collect DNA from at least 90 individuals. Our repository currently stores DNA from ~2000 individuals from 12 African and South-East Asian countries.

We have completed a pilot study spanning four genomic regions in 288 individuals from six populations. The regions contain alpha-globins (90kbp, chr16), beta-globin (110kbp, chr11), SLC4A1 (30kbp, chr17) and G6PD (20kbp, chrX). Automated variation detection pipeline has identified 19,000 putative SNPs, of which ~2300 have been verified manually. More than 50% of verified SNPs appear to be novel. Manual verification of oppositely fixed positions and putative insertion-deletion polymorphisms is ongoing.

Current commercially available genotyping platforms are too sparse for powerful GWA studies in malaria-endemic populations. In addition, markers used to successfully fine-map one population may fail to detect association in another population due to local variations in haplotype structure and low level of linkage disequilibrium (LD). Our project will help to identify tagging markers for fine-mapping studies and provide a rich source of information regarding genome structure in African and South-East Asian countries.

#### P10.52

##### multidrug resistance in iranian epileptic patients is not associated with g2677t/a polymorphism in the abcb1(mdr1)gene

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Purposes: P-glycoprotein 170 encoded by the multidrug resistance 1 (MDR-1) gene, exports various antiepileptic drugs out of the CNS, which leads to multidrug resistance.

The relationship between single nucleotide polymorphisms (SNPs) in the MDR1 gene and drug resistance in Iranians with epilepsy was evaluated in this study.

Methods: The SNP at nucleotide position G2677A/T in exon 21 of MDR1 gene was genotyped by PCR-RFLP and ARMS-PCR in 300 Iranian Individuals. Subjects were classified according to whether they had drug-resistant (n=100) or drug-responsive epilepsy (n=100) or healthy control (n=100).

Results: The frequencies of genotype and phenotype were compared between three groups. The results showed that the frequencies of genotype (G & A&T) and phenotype were not statistically significant

among the groups.

Our findings failed to prove an association between G2677A/T polymorphism in ABCB1 gene and drug resistance in Iranian epileptic patients

#### P10.53

##### Aetiology of mental retardation in Malta using clinical, cytogenetic, array CGH and molecular diagnostic techniques

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Introduction: Genetic aetiology of mental retardation is complex and includes chromosomal abnormalities, monogenic or polygenic conditions, microdeletion syndromes and epigenetic disorders.

Methods: A total of 380 individuals were studied using a diagnostic protocol based on dysmorphology and clinical assessment. Investigations included a routine karyotype, testing for cryptic chromosomal rearrangements using FISH and array CGH and DNA testing for *FMR1*, *MECP2* and other genes as indicated.

Results: A specific cause for the mental handicap was identified in 243 individuals (64%). These included a chromosomal abnormality in 86 (22%), fragile X syndrome in 7 (1.8%), Rett syndrome in 9 girls (2.4%), microdeletion syndrome in 4 (1%), recognizable syndromes in 43 (11%), neurological disorders in 25 (6.6%), metabolic conditions in 3 (0.8%), an environmental cause in 26 (6.8%) and autism spectrum disorder in 21 (5.5%). Subtelomeric screening in 73 families identified a microdeletion of 1pter and a t(7p:9p). Array CGH in 15 individuals with normal subtelomeric screens identified a 2MB duplication of 11q25, and a 6MB deletion of 22q12.

Conclusion: While clinical diagnosis and conventional techniques form the mainstay of investigation of individuals with mental retardation, molecular cytogenetics and array CGH proved important diagnostic tools increasing the diagnostic yield by 2%.

#### P10.54

##### Frequency of conserved extended haplotypes of the MHC region in Hungarian families

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Conserved extended haplotypes (CEH) or ancestral haplotypes (AH) are characteristic features of the major histocompatibility complex (MHC) region of the band 6p.21.3 on the short arm of chromosome 6. Although many studies performed on reference cell lines of homozygous individuals were reported recently, family studies with the updated CEHs suitable to establish their population frequencies are scarce. Therefore we performed such a study in 52 families in Hungary. Results obtained in 49 Caucasian and 3 Gipsy families were separately evaluated. Besides *HLA-A*, *-B* and *-Cw* as well as *HLA-DR1/DQ1* alleles, copy number polymorphism of the *C4A* and *C4B* genes and several SNPs encoded in the central (class III) MHC region (*RAGE* -429T>C, *Factor B S/F*, *HSP70-2* 1267A>G, *TNF* -308G>A and *LTA* 252A>G) were determined.

By analyzing 188 Caucasian haplotypes in Hungary we found 12 previously reported haplotypes in 53 copies. As expected, the most thoroughly studied haplotype AH8.1 or [B8, SC01, DR3] occurred most frequently (5.85%). In addition, 13 new, earlier not reported haplotypes in 32 copies were found in a frequency of at least 1% among the Hungarian chromosomes. Interestingly, a part of the *DRB1-DQB1* blocks of these novel haplotypes were of Central-Asian type, in accordance with the origin of the Hungarians. These findings indicate that conserved extended haplotypes are more frequent in the Caucasian population than it is expected therefore their detailed analysis would be required for disease-association studies of this region.

#### P10.55

##### Involvement of the modifier gene of a human Mendelian disorder in a negative selection process

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Identification of modifier genes and understanding their mechanism of action represent two major challenges in human genetics. *SAA1* is one of the few modifiers identified in humans. This gene is known to influence the risk of renal amyloidosis (RA) in patients with familial Mediterranean fever (FMF), an autoinflammatory disorder associated with mutations in *MEFV*. Indeed, the *SAA1* alpha homozygous genotype and the p.Met694Val homozygous genotype at the *MEFV* locus are two main risk factors for RA. Here, we investigated Armenian FMF patients and controls living in two neighboring countries: Armenia, where RA is frequent (24%) and Karabakh, where RA is rare (2.5%). The frequencies of p.Met694Val homozygotes were found to be similar in the two groups of patients. However, a major deficit of *SAA1* alpha homozygotes was found in Karabakhian patients as compared to Armenian patients ( $p=5.10^{-5}$ ), whereas, in the two control populations, genotype distributions at this locus were similar and complied with Hardy-Weinberg equilibrium (HWE). Most importantly, we observed deviations from HWE in the two groups of patients, and unexpectedly, in opposite directions. A population-based study revealed that the excess of *SAA1* alpha homozygotes in Armenian patients is readily explained by the recruitment of patients with severe phenotypes. In contrast, the deficit of alpha/alpha among Karabakhian patients revealed a negative selection against individuals carrying this genotype. This study, which provides new insights into the role of *SAA1* in the pathophysiology of FMF, represents the first example of deviations from HWE and selection involving the modifier gene of a Mendelian disorder.

#### P10.56

#### Research of monogenic hereditary ophthalmopathology of the Rostov region.

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Hereditary pathology of the eye comes up to about 30% in the general structure of eye disorders. Population of eight rural districts of the Rostov region was examined. Total size of investigated populations was 320925 persons (90% Russians). The research was conducted under the original examination protocol, providing for detection of more than 2500 various hereditary disorders (HD) and syndromes. The diagnostics was performed by physicians of different specialties possessing professional qualification of HD.

Families with monogenic hereditary ophthalmopathology (MHO) which constituted 36.8% of the total number of patients identified in this population. The prevalence of the whole MHO was 1:823 persons, including isolated forms 1:1408, and the prevalence of 1:1981 as a part of hereditary syndromes. Nosological spectrum of MHO in the Rostov region is notable for a great variety. The most numerous MHO groups were pathology of the retina, optic nerve and cataract. Virtually all hereditary forms of retina degeneration known as of today were described in the course of research, they make up 53% in the structure of retina and optic nerve diseases. Another nosological form determining a basic part of MHO burden in this population was autosomal dominant and autosomal recessive cataracts with microcornea. MHO detected in total was lower than in other rural populations of Russia.

#### P10.57

#### Myeloperoxidase gene G-463A polymorphism in the Southeastern Anatolia

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Myeloperoxidase (MPO) has been involved in the pathogenesis of several diseases through excessive production of reactive oxygen species (ROS) as well as through its genetic polymorphism.

The aim of this study was to determine Myeloperoxidase (MPO) gene G-463A region polymorphism in healthy population of Southeastern Anatolian region in Turkey. Also we compared the results according to the literature data if there is any difference between the healthy population of Southeastern Anatolian with the populations of different countries.

The subjects of this study were 150 unrelated healthy individuals. The genotyping was determined by polymerase chain reaction-restriction fragment length polymorphism method.

The genotype distribution were observed in healthy population: 4.6% in AA, 28.6% in AG and 66.6 % in GG. The frequency of A allele is 19 % whereas the frequency of G allele is 81 %.

The presence of the A and G allele frequencies in various populations such as China, USA, Australia, Brazil, Europe, France and Germany is similar to our results according to the published data. Nevertheless, it was understood that A allele frequency in the populations in Taiwan and Korea was lower than that of us while G allele frequency was higher and that there was a deviation from Hardy-Weinberg Equilibrium ( $p<0.05$ ) only in China and Germany populations.

#### P10.58

#### Genetic portraits of populations of Sakha (Yakutia): implications for the history of peopling of Northeast Eurasia

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Northeast Eurasia was colonized by modern humans at least 30 kya [Pitulko et al., 2004]. The consecutive archaeological cultures changing observed in the region is regarded as the effect of multiple waves of migrations from territories surrounding upper reaches of Enisei, Lake Baikal and Amur River basin. To elucidate the human colonization process in Northeast Eurasia mtDNA and Y chromosome lineages variation was analyzed in populations of the Republic Sakha (Yakutia) in the northeastern part of Russian Federation. Haplotypes of mitochondrial DNA were determined by sequencing of HVSI and analysis of 86 diagnostic sites of coding region in 694 individuals from 7 populations of Yakutia - Central, Vilyuy and Northern Yakuts, Evenks, Evens, Yukaghirs, Dolgans. The diversity of Y-chromosome lineages was studied in 318 men by typing 24 biallelic and 6 microsatellite loci of Y non-recombining portion. On the basis of 33 novel and 250 published complete mtDNAs sequences of haplogroups (hgs) CZ, D and R1, the topology of hgs C, Z, D4j, D4m, D4e, D5 and R1 was refined. We typed informative coding region markers defining distal twigs of the obtained trees in populations of Yakutia to mark out differences between them that could discriminate the different stages of colonization and to found the traces of ancient Paleolithic migrations in this area. To obtain a wider view of the entire region as a complete system, we analyzed our results in a broader context of the Eurasian mtDNA and Y-chromosomal variability.

#### P10.59

#### Mitochondrial DNA sequence variability in Bulgarian population

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Population mitochondrial DNA (mtDNA) variability is of special interest for forensic geneticists for correct statistical interpretation of probabilities of match. To date there is no profound study of mtDNA sequence variability among Bulgarian population. Our study is aiming at creating a referent mitochondrial Bulgarian database by direct sequencing of mtDNA control region (CR) in more than 300 unrelated individuals. Here we present our preliminary results.

MtDNAs of 94 unrelated Bulgarian individuals from different parts of Bulgaria were isolated with salt extraction technique. Two hypervariable segments (HVRI and HVRII) of approximately 870 nucleotides within the CR of mitochondrial genome were examined by direct automated sequencing on ABI3130xl.

Among 94 studied individuals were identified 89 different haplotypes. Seventy-three haplotypes were defined by 81 variable sites in HVRI and 64 haplotypes were defined by 43 variable sites in HVRII. Two novel, unreported so far, nucleotide changes were observed: 16233 A→T and 42 T→TG. Point mutation heteroplasmy was detected at five positions- 16093, 16287, 146, 185 and 298. Mitochondrial diversity (D) was estimated as 0.983 for HVRI, 0.980 for HVRII and 0.998 for both regions. These values, compared with other European populations (0.93-0.98), revealed very high genetic diversity of Bulgarian population. Random match probability, estimated for HVRI (2.7%), HVRII (3%) and both regions (1.2%), indicated that sequence analysis

of both HVRI and HVRII would be very informative for forensic practice in Bulgaria.

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#### P10.60

#### Mitochondrial Genome Diversity in Tungusic-speaking Populations (Even and Evenki) and Resettlement of Arctic Siberia After the Last Glacial Maximum

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The present study includes the Even/Evenki, hunters and reindeer-breeders, sampled from a few localities scattered across their vast geographic range encompassing low Yana-Indigirka-Kolyma in the west and the Sea of Okhotsk coast in the east. The mtDNA data show a very close affinity of the Even/Evenki with the Yukaghirs, typical reindeer hunters, dominating in extreme northeastern Siberia until the middle of 18th century but now being on the brink of extinction. We found that the majority of mtDNA diversity in the Tungusic-speaking populations was accounted for by Siberian-East Eurasian lineages C2, C3, D2, D3, D4-D9 and G1. The similarity in the haplogroup C and D mtDNA intrinsic variation between the Even and Yukaghirs populations is pronounced and indicates that the Even/Evenki harbor an essential portion of the ancestral Yukaghirs pool. The phylogeography of the D4-D9 point to an early Neolithic phase expansion initiated northward to the northern and eastern perimeters of former Beringia. Concerning unique D2\* lineage (Volodko et al. 2008), the network analysis encompassing four complete sequences, three of the Yukaghirs from the low Indigirka-Kolyma region and one of the Evenks from the upper reaches of the Aldan River would suggest that the founding haplotype (1935-8683-14905) for D2\* originated within western part of former Beringia. In the meanwhile, the core of the Even/Evenki mtDNA pool residing in the midst of the Yukaghirs ancient territory would represent a recent amalgamation of the remnants of the Yukaghirs and northern Tungusic-speakers (Even/Evenki) originated in the mid-Amur region.

#### P10.61

#### The monitoring results of the newborn congenital development defects (CDD) in Rostov region.

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Children CDD monitoring in Rostov region has been taking since 01/01/2000. Taken research enabled made possible to identify common CDD rate and newborn strict account anomalies' frequency, which were  $15.32 \pm 0.37\%$  and  $8.66 \pm 0.28\%$  respectively. Multiply congenital development defects (MCDD) frequency was  $3.44 \pm 0.18\%$  (1:291). CDD structure analysis displayed that MCDD keep second place after heart anomalies and form 22.47% from general diseases quantity. Analysis which was taken according with the prevalence rate, displayed that isolated CDD form were 77.53%, MCDD - 12.99%, chromosomal anomalies - 9.48%, according with etiology - multifactorial CDD - 82.50%, chromosomal anomalies - 9.48%, monogenic diseases - 8.02%. The frequencies of CDD according with etiology were made up: multifactorial -  $12.64 \pm 0.33\%$ , chromosomal -  $1.45 \pm 0.11\%$  and monogenic -  $1.23 \pm 0.10\%$ . MCDD structure was presented by unspecified complexes - 48.18%, chromosomal anomalies - 42.19%, monogenic syndromes MCDD - 8.85%. They are including autosomal dominant (6.77%), autosomal recessive (1.56%), X-linked (0.52%) and unknown etiology syndromes (0.78%). Taken genetic consultation of families made possible to diagnose 22 monogenic syndromes. Children overall chromosomal anomalies rate in Rostov region formed  $1.45 \pm 0.11\%$  (1:689), among them which consisted of Down syndrome, Patau syndrome, Turner syndrome, Edwards syndrome, structural chromosomal aberrations (18q-) and (5p-). Patau syndrome and Edwards syndrome were attended by heavy forms of congenital heart anomalies, which lead to high infant mortality affected them in the first day after born, and according to this, low detectabilities of this syndromes in Rostov region. The received results are conformed to the Russian Federal Register's data and EUROCAT.

#### P10.62

#### High-resolution melting analysis for the genotyping of an *Alu* insertion/deletion polymorphism at the myotonic dystrophy type 1 locus

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Myotonic dystrophy type 1 (DM1) is the most common autosomal dominant neuromuscular disorder of adults associated with unstable expansions of a (CTG)n repeat tract in the 3'-untranslated region of the *DMPK* (Dystrophia Myotonica Protein Kinase) gene, on chromosome 19q13.3. Haplotype analyses demonstrated a complete allelic association of DM1 causing alleles with several intragenic and extragenic polymorphisms among which the *Alu* insertion/deletion polymorphism located 5 kb telomeric to the (CTG)n repeat tract is one of the most studied. In patients of European and Asian ancestry the larger allele (*Alu*+) was found to be in complete linkage disequilibrium with DM1 causing alleles. The aims of our study were to design a simple, rapid and reliable method for genotyping the *Alu* insertion/deletion polymorphism and to study the status of this polymorphism in conjunction with the number of (CTG)n repeats on DM1 and healthy chromosomes in Slovak population. For rapid genotyping of this locus we successfully combined the previously described three-primer PCR amplification protocol (with modified primers) and high-resolution melting (HRM) analysis using a 96-well LightScanner and a fluorescent DNA binding dye. In sample of Slovak DM1 patients all of the identified DM1 expanded alleles were found to be in association with the *Alu*(+) allele. This fact together with the finding of all large-sized normal alleles being also on the *Alu*(+) background is consistent with the hypothesis that alleles with more than 18 CTG repeats may form a pool of unstable alleles that may constitute a reservoir for recurrent DM1 mutations.

#### P10.63

#### A retrospective study of neural tube defects

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Neural tube defects (NTDs) are one of the most common birth defects, occurring in approximately one in 1,000 live births, caused by a combination of multiple genes and multiple environmental factors. We present an overview of 315 NTDs cases registered in Clinical Hospital of Obstetrics and Gynecology from Oradea (2,54% of babies born alive) with a relatively constant distribution along the studied interval 1984-2008. The most frequent NTDs were represented by hydrocephaly (37,46%), spina bifida (30,15%) and cephalocele (17,46%). Most cases come from mothers of 20-25 years of age and it was their first pregnancy in 52,06% cases. We notice a higher incidence of these abnormalities in the female sex (55,23%). 55,87% of cases were born prematurely. Most of the NTDs babies born alive had a good clinical condition at birth, with an Apgar score of 10-7. Hydrocephaly was the first cause of neonatal death in 54,92% of the cases.

The genetic evaluation NTDs cases concluded a possible multi-factorial polygenic heredity in most of the cases, in absence of a suggestive family history the monogenic forms being only presupposed. In cases that associated NTDs with multiple anomalies we suspected a possible chromosomal heredity. The maternal gestational diabetes was incriminated in 1,6% cases. Maternal hyperthermia during the embryogenesis was suspected in 1,2% cases. Aminopterin and the valproic acid, teratogenic factors associated for certain with NTDs weren't identified in the preconceptional history and in the first trimester of pregnancy in none of the cases evaluated retrospectively or prospectively.

#### P10.64

#### The prevalence of 657del5 mutation of the NBN gene in the Lviv Region of Ukraine

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Nijmegen breakage syndrome (NBS) is a rare chromosomal instability disorder, characterised by microcephaly, facial dysmorphism, growth retardation, immunodeficiency, hypersensitivity to radiation and a high

predisposition to lymphoid tumors. Most of the NBS patients are of Slavic origin and carried a major founder mutation 657del5 of the NBN gene. The prevalence of the 657del5 in the West Ukraine was previously studied by R. Varon et al. (2000) and frequency of heterozygotes was estimated as 1:182, expected NBS cases - 1:133 000. Since 1999 and till 2009 the 30 NBS cases were diagnosed in Ukraine, half of them - in the Lviv region of the West Ukraine. The purpose of this study was to evaluate the prevalence of 657del5 mutation of the NBN gene in Lviv population upon a base of verified NBS cases. The total number of 511598 children were born in Lviv region in 1989-2007 years and 11-years period was marked by NBS case appearance: 1989 (1), 1990 (1), 1992 (2), 1994 (1), 1998 (2), 2001 (1), 2002 (1), 2003 (2), 2004 (1), 2005 (1), 2007 (2). Thus, in the time span of 2 years, 1-2 new NBS cases are expected to be born. The frequency of Nijmegen breakage syndrome in Lviv population is estimated as 1 per 13640-34106 neonates, whereas the prevalence of 657del5 heterozygotes is approximately 1 per 58-95 neonates. The high frequency of heterozygous carriers of NBN founder mutation in Lviv population may contribute to cancer frequency in the West Ukraine.

#### P10.65

#### The spectrum of GJB2 (connexin-26) mutations in patients with nonsyndromic hearing loss: high prevalence of the allelic variant V37I (109G>A) in Asian populations of Sakha (Yakutia)

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Congenital hearing loss is one of the frequent sensory defects affecting 1-3 in 1000 newborns, and 50% of these cases are hereditary determined. Nonsyndromic hearing loss (NSHL) accounts approximately 80% of hereditary deafness cases. GJB2 mutations are implicated as a major cause for the development of NSHL in majority of populations. We studied GJB2 mutation spectrum in patients with NSHL in Sakha Republic (Yakutia) (East Siberia, Russia). A total of 66 patients with NSHL of different ethnic affiliation (Caucasians and Asians) were analyzed by PCR-SSCP and further sequencing of GJB2 gene. The molecular screening of V37I in 6 populations of Yakutia (Yakuts, Dolgans, Evenks, Evens, Yukaghirs and Russians) was performed using allele-specific amplification PCR method.

GJB2 mutations were found in 50.0% of Caucasian and 9.2% of Asian patients chromosomes. We identified 3 different deletions 35delG, 312del14, 333-334delAA known as recessive and 5 different missense mutations V27I, M34T, V37I, R127H, E114G which pathogenic role is controversial. One of the common allelic variants in Asian patients is V37I (45% of all mutant chromosomes). Molecular screening of V37I in 6 populations of Yakutia showed that it was one of the common allelic variant in studied populations. We found the V37I allele in Turkic-speaking populations of Yakuts, Tungusic-speaking populations of Evenks and Evens and Slavic-speaking ones of Russians. V37I was not detected in populations of Dolgans and Yukaghirs. High prevalence of V37I in populations of Yakutia may be a result of common founder effect.

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#### P10.66

#### The comparative analysis of hereditary skeletal disorders' spectrum in Rostov region

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Hereditary skeletal disorders (HSD) are a large heterogeneous group of genetic diseases with different prevalence rate in populations. A cumulative international incidence of at least 1:5000 newborns has been estimated (in most cases, it was estimated by cumulating information from the different publications). The following ten syndromes: Marfan syndrome - 30:100,000; Arthrogryposis multiplex congenita

- 30:100,000; Polydactyly, preaxial - 25:100,000; Syndactyly, type 1 - 25:100,000; Ehlers-Danlos syndrome, type 3 - 12.5:100,000; Osteogenesis imperfecta - 6.5:100,000; Achondroplasia - 4.5:100,000; Epiphyseal dysplasia multiple - 5:100,000; Acrocephalosyndactyly - 4.6:100,000; Diastrophic dwarfism - 3.5:100,000 were the most frequent HSD (isolated and syndromic forms) in the world. 320925 people of eight areas of Rostov region were examined. The research was made taken under the RCMG RAMS protocol, which, according to OMIM, provides more than 2500 genetic diseases diagnosis. Then families with HSD were selected from the cite data. In the results 336 patients from 226 families were detected with HSD. The total prevalence rate of HSD in Rostov region was 1:950. The following ten syndromes: Ehlers-Danlos syndrome, type 3 - 15.6:100,000; Marfan syndrome - 5:100,000; scoliosis, idiopathic - 5:100,000; Polydactyly, postaxial - 4.7:100,000; Noonan syndrome - 4:100,000; Syndactyly, type 1 - 3.7:100,000; Osteogenesis imperfecta - 3.1:100,000; Achondroplasia - 2.8:100,000; Syndactyly, type 2 - 2.8:100,000; Polydactyly, preaxial - 2.5:100,000 were the most frequent HSD in the Rostov region. Diversity and incidence of the most frequent HSD differs from the world data. The further research will enable to reveal the reasons of this regional HDS spread peculiarities.

#### P10.67

#### Epidemiological aspects of Osteogenesis Imperfecta in Bashkortostan Republic of Russia

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Osteogenesis imperfecta (OI) is a clinically and genetically heterogeneous disorder of connective tissue characterized by brittle bones, blue sclerae, short stature, bone deformity, hearing loss and dentinogenesis imperfecta. 95 patients with OI are identified from 90 families the Republic of Bashkortostan (RB). The prevalence of OI is estimated at 1 per 45000, whereas in other countries of the world it is estimated to be 1:10000-1:30000. Our results show that OI is extended irregularly in the RB. The disease is detected in 29 from 54 administrative districts and in 12 from 21 cities. The incidence of OI varies from 0.42:100000 to 17.8:100000. The distribution of patients by the sex composition is as follows 52males and 43 females. Epidemiological investigations based on the ethnic composition of 95 patients with OI scattered are as follows: Tatars - 29.4%, Russians- 23.1%, Bashkirs - 18.9%, 6.2% from Armenians, Maris, Ukrainians, Chuvashes and 22.4% are metis. 70 from 95 cases have blue sclera (34males and 36females). 16 patients have autosomal dominant and 79 have autosomal recessive inheritance pattern. More than 10 fractures during their lifetime had 39 patients, multiple fractures - 33 patients and 23 patients didn't have fractures. The distribution of patients with OI by the age composition is congenital 22 cases (26%), from 1 to 7 years old 35 patients (42.8%) and from 8 and older 27 cases (32.3%). The irregular prevalence of OI in RB, apparently, occurs randomly as absence of patterns associated with local concentration incidents with OI.

#### P10.68

#### Study of two common P53 gene mutations in gastric cancer using (PCR-RFLP) in a Province of Iran

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Background and aim: Gastric cancer is the most common cause of cancer death world wide after lung cancer. Genetic factors including oncogenes and tumor suppressor genes are always involved in progression of this cancer. The P53 tumor suppressor gene is believed to have a broad role in the cell such as programmed cell death and stop cell replicating damaged DNA which has been summarized on the guardian of the genome. This study aims to determine the frequency of two common P53 gene mutations using PCR-RFLP in gastric cancer in a Province of Iran

Methods: This descriptive - lad based study describes the mutation analysis of paraffin embedded gastric samples from 38 patients in a Province of Iran. We have investigated the frequency of P53 gene mutation in exons 7 and 8 by PCR-RFLP to detected alteration in two common hot spots in codon 248 and 282.

Results: We determined no mutation in P53 gene hot spots in codon 248 and 282.

Conclusion: We conclude that association of P53 gene mutations with gastric cancer is very low in Chaharmahal Va Bakhtiari, a Province of Iran. However we have examined only 38 gastric samples and more samples need to be investigated to reveal the contribution of P53 gene mutation in causing gastric cancer in this province. Also it is necessary to study the entire coding region and promoter of the gene in patients from different population and ethnic groups.

#### P10.69

##### The PPARC1A G1444A polymorphism in Lithuanian professional athletes and the general population

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The peroxisome proliferator-activated receptor gamma coactivator-1 (*PGC1A*) is involved in regulation of fatty acid oxidation, skeletal muscle fiber type specificity, and gluconeogenesis. The prevalent G1444A (rs8192678) variant in *PGC1A* was shown to be associated with traits of the metabolic syndrome. Moreover, it is unclear whether it influences human physical performance. The hypothesis of the present study was that frequency of the minor [A] allele at the *PGC1A* locus is lower in Lithuanian professional athletes than in the general population of Lithuania. We tested genotypes and allele frequencies of this SNP in athletes ( $n = 551$ ; mean age:  $17.7 \pm 5.3$  years) and the control group of general population of Lithuania ( $n = 97$ ; mean age:  $31.3 \pm 13.5$  years). Genotyping was performed by PCR and restriction enzyme digestion. Genotypes of athletes were identified as [G/G] 52.1%, [G/A] 42.1%, [A/A] 5.8% ( $\chi^2=2.83$ ,  $p=0.09$ ) and the genotypes in the population samples were [G/G] 42.2%, [G/A] 37.8% and [A/A] 20.0% ( $\chi^2=3.79$ ,  $p=0.055$ ). The frequency of the minor [A] allele was significantly lower in athletes than in controls (26.9% vs. 38.9%;  $P = 0.01$ ). *PGC1A* [A/A] genotype is less frequent in Lithuanian athletes in comparison to the general population (5.8% vs. 20.0%;  $P=0.005$ ). The results of the present study imply that the *PGC1A* [G] allele of the [G/G] genotype is more common in the professional athlete group than in the general population of Lithuania. In conclusion, the present findings suggest that there is an association between *PGC1A* G1444A polymorphism and physical performance in Lithuanian athletes.

#### P10.70

##### Analysis of 10 autosomal DNA markers in Bashkir population

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The purpose of our work was investigation of genetic differentiation between ethno-geographic bashkir groups. Genetic structure of Bashkir subpopulations has been studied based on analysis of 10 autosomal DNA markers (diallelic and multiallelic): *CCR5Δ32*, *ACE*, *D7S23(KM19)*, *STR/TH01*, *STR/FABP*, *STR/IVS6a*, *VNTR/PAH*, *VNTR/ApoB*, *VNTR/DAT1*, *VNTR/eNOS* (53 alleles). The total number of samples, which was more than 800 individuals belonging to three ethno-geographic bashkir groups: south-eastern (Burzyansky, Abzelilovsky, Baimaksky and Kugarchinsky districts), north-eastern (Arkhangelsky and Salavatsky districts) and north-western (Askinsky district) (7 subpopulations), were analyzed. Analysis of allele's frequency of autosomal DNA markers in Bashkir subpopulations shows considerable genetic differentiation between Bashkir subpopulations. The highest level of genetic diversity in diallelic system was established on locus *ACE*,  $H_{obs} = 0.5278$ , in multiallelic system - on locus *STR/TH01*,  $H_{obs} = 0.7520$ . The level of genetic differentiation between bashkir subpopulations is higher than in Udmurt and Chuvash populations ( $F_{ST} = 0.008$ ). The analysis of dendograms, based on correlations between the matrix of genetic distances, and multidimensional scaling analysis showed that south-eastern and north-eastern ethno-geographic groups of Bashkir

are genetically closer to each other than to north-western group. Our findings are consistent with evidences on Bashkir ethnogenesis and historical facts.

#### P10.71

##### Analysis of three Microsatellite Markers (D5S818, D7S870 and D13S317) in Romanian population and their genetic relationship with other European populations

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We report on analysis of 3 different DNA-polymorphisms (Microsatellites D5S818, D7S870 and D13S317) in a sample of 200 individuals from Bucharest, Romania, as a part of a more complex study, in order to elucidate the genetic structure of Romanian population and to show their genetic relationship with other European human populations.

Genomic DNA was isolated from whole blood samples and multiplex PCR amplified by AmpF/STR Profiler Kit (ABI). Allele assignment was performed by capillary electrophoresis by ABI 3100 Analyzer. Our results were compared with similar data of a Romanian population sample from Prahova Valley and other European human populations.

The genetic relationship between populations was evaluated based on both Nei's genetic distance and Principal Component Analysis (PCA) by Phylip Package (version 3.6) and Statistical Package for the Social Sciences Software.

Our results revealed no significant difference in allele frequencies of the three microsatellite markers between the panmictic population of Bucharest and the slight isolated population from Prahova Valley.

Genetic distance analysis and PCA showed closer genetic kinship to Greek population, as well as Slavic population from Poland.

Intercultural exchanges and intense trading activities between old human populations from Romania (Thracians) and Greek population groups, who established colonies on the west coast of the Black Sea (nowadays East-Romania) during the 7th-8th centuries, may explain our findings.

The Slavic influence may be the result of migrations of Slavic groups across the Carpathian-Danube regions during the 6th-9th centuries.

These data can also be used for paternity and forensic analyses in Romanian population.

#### P10.72

##### High sequence variability of exon 2 of runt-related transcription factor 2 (RUNX2) in four Siberian populations

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The runt-related transcription factor 2 (RUNX2) is the principal osteogenic master switch, which acts as regulator of osteoblast differentiation and skeletal morphogenesis. Mutations of RUNX2 gene were shown to cause cleidocranial dysplasia (OMIM 119600). It's also believed that this gene is a candidate for osteoporosis. To date, a few reports about polymorphisms in the RUNX2 gene have been published describing population prevalence of repeat length variants in glutamine and alanine stretches and nearby SNPs. We employed PCR-RFLP and DNA sequencing to screen genetic variations within the exon 2 of RUNX2 gene in DNA samples of Russians ( $n=96$ ), Yakuts ( $n=96$ ), Tuvians ( $n=96$ ) and Buryats ( $n=96$ ). In total of 15 chromosomes with glutamine stretch variants (16Q, 30Q and 32Q) were detected in our study. Two mutants (16Q) were found among Russians. One 32Q allele and one 30Q allele were identified in Yakutia and Tuva, respectively. The most abundant sequence variability was registered in Buryatia. From the 96 individuals genotyped, there were a total of 11 glutamine tract variations, including ten 32Q alleles and one 30Q allele. All RUNX2 variants were heterozygous for a mutant allele and a wild type allele. We revealed two Tuvians with novel variant NM\_001924630.2: c.467C>T (NP\_001019801.2: p.A156V). The frequencies of alleles and genotypes of common 18 base pair deletion of polyalanine tract and rs6921145:G>A were also established in our study. These results suggest that there is considerable sequence variability of exon 2 of RUNX2 gene in ethnically diverse Siberian populations.

## P10.73

## Genetic disorders in Saudi Arabia- An update

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Saudi Arabia, is the largest Arab country and has an estimated population of around 27.6 million. The family and tribe are the basis of the social structure and Saudis are cognizant of their heritage, their tribe, and their extended and nuclear family. Consanguinity and other factors, including environmental factors, have played a significant role in accumulating genetic disorders, some very rare ones, at a higher frequency in the Saudis. We conducted three National studies over a period of twenty years to screen the entire country for common single gene disorders (including sickle cell gene,  $\alpha$ - and  $\beta$ - thalassaemia, glucose-6-phosphate dehydrogenase deficiency) and multifactorial disorders (including diabetes mellitus, obesity, and hypertension). Over 60,000 samples were screened and gene frequencies of these disorders were obtained. In addition, studies conducted in other institutions reported several inborn errors of metabolism, chromosomal, mitochondrial and somatic cell disorders (cancers). Several disorders, rare in other populations, occur at a high frequency in the Saudi. The genetic basis of several of these disorders has been unveiled and very interesting picture has emerged for the common disorders, where mutations specific to Saudis and rare in other populations form the basis of several of the common disorders. The natural history of several of the disorders has been investigated and a wide range of clinical diversity has been identified. Steps have been adopted towards primary prevention. This paper will present a comprehensive coverage of the present status of genetic diseases in Saudi Arabia and steps adopted towards control and prevention.

## P10.74

## Ancestral origin of pure repeat expansions and CAA interrupted alleles in spinocerebellar ataxia type 2 (SCA2)

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The spinocerebellar ataxia type 2 (SCA2) is an autosomal dominant neurodegenerative disease characterized by gait and limb ataxia. This disease is caused by the expansion of a (CAG)n located in the ATXN2, that encodes a polyglutamine tract of more than 34 repeats. Lately, alleles with 32-33 CAGs have been associated to late-onset disease cases. Repeat interruptions by CAA triplets are common in normal alleles, while expanded alleles usually contain a pure repeat tract. To investigate the mutational origin and the instability associated to the ATXN2 repeat, we performed an extensive haplotype study and sequencing of the CAG/CAA repeat, in a cohort of families of different geographic origins and phenotypes. Our results showed (1) CAA interruptions in ATXN2 alleles, regardless of its pathogenic nature, and that (2) CAA interrupted alleles in the range 33-44 repeats shared an ancestral haplotype with pure expanded alleles; (3) an intragenic SNP-based haplotype, C-C, common to all SCA2 families regardless of its interruption pattern, origin or phenotype; and (4) higher genetic diversity in European SCA2 families, suggesting an older European ancestry of SCA2. In conclusion, we found a shared ancestral ATXN2 haplotype for pure and interrupted expanded alleles, with strong implications in mutation diagnosis and counseling. Our results indicate that interrupted alleles, below the pathological threshold, may be a reservoir of mutable alleles, prone to expansion in subsequent generations, leading to full disease mutation

## P10.75

## Allele frequencies of eight short tandem repeat loci in East Azerbaijan province population

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Short tandem repeats (2-6 bp) have become wide-spread in their use by the forensic DNA typing, Paternity testing, gene mapping, and diagnosis of hereditary disease. Allele frequencies for 8 STR loci (D16S539, D8S1179, D5S818, D13S317, F13B, MTHO1, TPOX and FES/FPS) were determined in the samples of 218 un-related volunteer of East Azerbaijan province population using PCR and subsequent poly-acrylamid gel electrophoresis and silver staining.

Regarding to the results, Among 8 STR loci, heterozygosity of D16S539, D5S818, D8S1179, D13S317, F13B, FES/FPS, MTHO1, TPOX respectively were 0.8213, 0.8188, 0.7883, 0.8062, 0.7442, 0.7397, 0.7834, 0.6769. No deviation from Hardi-winberg equilibrium was observed. D16S539 with 0.8213 heterozygosity is the most informative marker and TPOX with 0.6769 heterozygosity was the least informative marker on target group. Therefore except for TPOX all mentioned markers could be used for forensic DNA typing and paternity test of East Azerbaijan population.

## P10.76

## Forensic value of 10 STR loci in the entire region of Turkey population and comparisons to other ethnics groups or areas

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Allele frequencies of the 10 STRs loci (D16S539, D2S1338, D3S1358, vWA, D18S51, D21S11, D8S1179, D19S433, FGA, TH01) included in the AmpFISTR SGM Plus kit were obtained from different biological materials of sample of 100 unrelated individuals in the entire region of Turkey. Chi-square test showed that all STR loci agreed with Hardy-Weinberg equilibrium. The population genetic data were compared with the previously publishing population data of Turkish and other ethnic groups or areas. The results of present study suggest that 10 STR loci with its high combined PD values (0.999999999998) seem to be a useful system for the cases in forensic identifications.

## P10.77

## Estimation of SMN1 Deletion Carrier Frequency in the Iranian Population based on Quantitative Analysis

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Spinal muscular atrophy (SMA) is a common autosomal recessive neuromuscular disorder caused by mutations in the *survival motor neuron 1* gene (SMN1). Carrier frequency studies of SMA have been reported for various populations. Although no large-scale population-based studies of SMA have been performed in Iran, previous estimates have indicated that the incidence of autosomal recessive disorder partly because of the high prevalence of consanguineous marriage is much higher in the Iranian population than in other populations. In this study, we used a reliable and highly sensitive quantitative real-time PCR assay with SYBR green I dye to detect the copy number of the SMN1 gene to determine the carrier frequency of SMA in 200 healthy unrelated, non consanguineous couples from different part of Iran

To validate the method in our samples, we determined the  $\Delta\Delta Ct$  ratios of patients with homozygous deletion (0.00) and hemizygous carriers (0.29 to 0.55). The  $\Delta\Delta Ct$  ratios in 10 of 200 normal individuals were within the carrier range of 0.31-0.57, estimating a carrier frequency of 5% in the Iranian population. Our data show that the SMA carrier frequency in Iran is higher than in the European population and that further programs of population carrier detection and prenatal testing should be implemented.

**P10.78****Retrospective epidemiological study of spinal muscular atrophy in Slovenia**

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**Introduction:** Spinal muscular atrophy (SMA) is one of the most common autosomal-recessive neuromuscular disorder. It has a prevalence of between 1 in 6000 and 1 in 10,000 live births in Caucasian populations. The epidemiology of SMA in our country was not published yet. In retrospective study we examined patients with clinical diagnosis of SMA in order to estimate the prevalence of SMA.

**Material and Methods:** Molecular genetic analysis was performed for patients with a clinical diagnosis of SMA, whose blood samples were sent to our laboratory during the 10 years period (from 1998 until 2008). PCR/RFLP analysis was used to detect homozygous deletion of the *SMN1*. MLPA analysis was found to be an efficient method for detecting copy numbers of *SMN1* and *SMN2*.

**Results:** After screening by PCR/RFLP analysis 75 out of 158 patients had homozygous *SMN1* deletion. Exons 7 and 8 were homozygously deleted in 58 patients (77%), while 17 (23%) showed deletion of only exon 7. In one SMA patient, MLPA revealed 1 copy of *SMN1*. In one family, 2 siblings with SMA and 1 non-affected showed only homozygous deletion of *SMN2*. Additionally, homozygous deletion of *SMN2* was detected in the mother, whereas her daughter's lack both copies of *SMN1*. The prevalence of SMA was estimated at 3.94/10<sup>5</sup> on September 30<sup>th</sup> 2008.

**Conclusions:** The prevalence is comparable to previously reported data in other Caucasian populations. Due to complexity of SMA genetics, testing of additional family members should be suggested and offered when appropriate.

**P10.79****Age- and sex- related reduction of relative telomere length over ten years in the population-based Bruneck study: Application of a high-throughput real-time PCR genotyping assay**

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**Background:** Telomeres play a key role in the maintenance of chromosome integrity and stability. Telomere length is linked to age-related diseases, with shorter telomeres associated with an increased probability of mortality from infection or heart disease. Our aim was to determine the decrease rate of telomere length over a time period of ten years and whether this decrease rate was influenced by age, sex and smoking behaviour.

**Research design and methods:** We compared relative telomere lengths (RTL) in 510 sample pairs from the longitudinal population based Bruneck study, which were collected in 1995 and ten years later in 2005. RTL were determined by a high-throughput real-time genotyping assay and by applying various mathematical models.

**Results:** Rate of change in RTL was highly variable among individuals. Mean telomere length decreased over ten years by 20.4% (95% CI: 16.8 - 24.1; p<0.001). The RTL attrition rate was highly correlated with the starting RTL in 1995 (r=0.743, p<0.001) and showed differences between males and females. By contrast, smoking behaviour seemed to have no influence on telomere lengths.

**Conclusions:** Our findings underscore the complexity of telomere dynamics and highlight the importance of taking measurement errors into consideration when interpreting uncommon findings. Our methodology proved to be a reliable and replicable tool for a rapid determination of relative telomere length with a low amount of input DNA. The most striking observation was that age-dependent RTL shortening was proportional to RTL at baseline examination.

**P10.80****The index of endogamy in the Buryatia Republic**

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The analysis of marriages which have been made in 1961-1965, 1977-1981 and 1997-2001 (further - the first, second, third generations) in city Ulan-Ude is lead. It is analysed more than 56 % from number of the registered marriages in each of generations.

For Russian population of Ulan-Ude on three generations of the index of endogamy - 7,36, 15,71 and 25,75 % consistently in three generations is marked. The index of endogamy for Russian population of Russia in 90th years are described at research of small cities of Krasnodar region and the Kostroma region, and during later period - for urban population of the Rostov and Tomsk regions. Alongside with the data received for Russian population of the Ulan-Ude, dynamics of the index of endogamy for the Buryat also had a positive gain, however sizes of this parameter were lower and have made 0,18, 0,29 and 2,12 % in three generations. The index of endogamy of the Buryat in the third generation is lower in comparison with city indigenous population of Udmurts and Maris. Thus, for aborigines Buryatiya (Buryat) and Russian population, living in the city of Ulan-Ude, the shown distinctions in the index of endogamy.

**P10.81****The prevalence of hereditary eye pathology in two regions of Kirov area of the Russian Federation**

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The role of hereditary pathology in etiology of eye disease in different populations and ethnic groups according to the literature makes from 20 to 45 %. To determine and study the prevalence of hereditary ophthalmic pathology (HOP) was organized the total inspection of the population of two regions of the Kirov area (Svechinsky and Shabalinsky). The total number of population is consist 21858 persons (90 % of population are Russian). The examination of patients was conducted by a group of doctors of different specializations, focused on a hereditary pathology. Prevalence HOP (autosomal-dominant, autosomal-recessive and X-links) in Svechinsky area is account 1:228 the person; in Shabalinsky - 1:287. The spectrum of HOP detected in 2 regions was formed by 31 diseases.

Among the isolated HOP following diseases (14 forms) were registered: different forms of congenital cataracts, congenital glaucoma, hereditary dystrophy of a cornea, iris coloboma, congenital nystagmus, bilateral ptosis, Best macular dystrophy, Wagner syndrome, choroideremia, optic atrophy with congenital myopia. HOP as a part of hereditary syndromes was found in 17 forms: Usher; Gronblad-Strandberg; Sturge-Weber; Aarskog; Treacher-Collins-Franceschetti; Marfan; Holt-Oram; Noonan; Williams-Beuren; albinism oculocutaneous; microcephaly with telecanthus, hypertelorism; camptodactyly with blepharophimosis; oligodontia with congenital iris coloboma, microphthalmia; relative deafness with a congenital cataract; congenital ataxia with defects of eyes; mental retardation with ptosis and a converting strabismus. Congenital cataracts were detected as the most frequent form (41 %), presented by various clinical and genetic variants. The significant familial polymorphism was noted in many cases. The most frequent type of inheritance was autosomal-dominant.

**P10.82****Tumor necrosis factor - alpha gene promoter polymorphisms in healthy population of Southeastern Anatolian**

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The production of cytokine varies among individuals and correlates with the polymorphism of cytokine genes. Tumor Necrosis Factor - alpha (TNF- $\alpha$ ) is an important cytokine that has been implicated in the pathogenesis of a number of diseases.

The aim of this study is to determine TNF- $\alpha$  promoter (-308,-238 and -857) polymorphisms in healthy population of Southeastern Anatolian region in Turkey. Also we compared the results according to the literature data if there is any difference between the healthy population of Southeastern Anatolian with the populations of different countries.

The subjects of this study were 150 unrelated healthy individuals. The

genotyping was determined by PCR-RFLP methodology. The genotype distribution for -308 region was observed: 1.3% in AA, 35.3% in AG and 63.4% in GG. The genotype distribution for -238 region was observed in our population: 0% in AA, 4.7% in AG and 95.3% in GG. Also the genotype distribution for -857 region were observed in our population: 12.7% in TT, 30.7% in TC and 56.6% in CC. We compared our results with the literature data of healthy populations in 13 different countries. No deviation for -308 and -238 regions from Hardy-Weinberg Equilibrium (HWE) were observed in our population ( $p > 0.05$ ) whereas deviation for -857 region from HWE was observed ( $p < 0.05$ ). These results show that these deviations occur due to the fact that our region is the transition region of migrations. This is the first study in Turkish healthy population for the TNFa promoter (-308,-238 and -857) polymorphisms.

#### P10.83

#### The *USH2A* c.2299delG mutation: dating its common origin in Southern Europe population

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Usher syndrome type II is the most common form of Usher syndrome. Although 3 genes are known as disease causing, *USH2A* is the major involved gene. It encodes two isoforms of the protein usherin. This protein is part of an interactome that plays an essential role in the development and the function of the stereocilia of inner ear hair cells. In the photoreceptor, usherin is located at the periciliary region between extern and inner segments. This gene contains 72 exons over a region of 800 kb. Although numerous mutations have been described, the c.2299delG mutation is the most prevalent in several populations. Its ancestral origin was previously suggested with the identification of a core haplotype restricted to 250 kb in the 5' region of the gene. Because we extended the haplotype analysis over the 800 kb region with a total of 14 intragenic SNPs, we could define 10 different c.2299delG haplotypes showing a high variability but with the conservation of the previous described core haplotype. An exhaustive c.2299delG/control haplotypes study suggests that the major source of haplotype variability in *USH2A* gene is recombination. Furthermore, we have found twice amount of recombination hotspots in the 500 kb 3' region of the gene, explaining the higher variability observed in this region comparing to the first 250 kb. Our data confirm the common ancestral origin of the c.2299delG mutation and suggest that it arose 5,500-6,000 years ago.

#### P10.84

#### A complex selection signature at the human AVPR1B gene

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The vasopressin receptor type 1b (AVPR1B) is mainly expressed by pituitary corticotropes and it mediates the stimulatory effects of AVP on ACTH release; common AVPR1B haplotypes have been involved in mood and anxiety disorders in humans, while rodents lacking a functional receptor gene display behavioral defects and altered stress responses.

Here we have analyzed the two exons of the gene and the data we present suggest that AVPR1B has been subjected to natural selection in humans. In particular, analysis of exon 2 strongly suggests the action of balancing selection in African populations and Europeans: the region displays high nucleotide diversity, an excess of intermediate-frequency alleles, a higher level of within-species diversity compared to interspecific divergence and a genealogy with common haplotypes separated by deep branches. This relatively unambiguous situation coexists with unusual features across exon 1, raising the possibility that a nonsynonymous variant (Gly191Arg) in this region has been

subjected to directional selection.

Although the underlying selective pressure(s) remains to be identified, we consider this to be among the first documented examples of a gene involved in mood disorders and subjected to natural selection in humans; this observation might add support to the long-debated idea that depression/low mood might have played an adaptive role during human evolution.

#### P10.85

#### Estimating the heritability of vitamin b12 levels: a study of adult female twins

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**Background:** Vitamin B12 (cobalamin) is an essential cofactor involved in one carbon metabolism (remethylation of homocysteine to methionine) and in the metabolism of branched chain amino acids. Currently the extent of the genetic and environmental influences on vitamin B12 levels has not yet been determined. Our aim was to determine the first heritability estimate for vitamin B12 levels in an adult female twin population.

**Methods:** We estimated the heritability of vitamin B12 levels in 1063 female twin pairs (262 monozygotic twin pairs and 801 dizygotic twin pairs), aged 18 to 80 years from the TwinsUK Adult Twin Registry. Structural genetic modeling was used to determine the influence of genetic and environmental factors on vitamin B12 variation.

**Results:** Genetic factors showed to account for 52% (95%CI, 45-58%) of vitamin B12 variation. The variance in vitamin B12 levels was explained by additive genetic and non-shared environmental factors, with the additive genetic variance estimated to 52% (95%CI, 45-58%) and the non-shared environmental variance to 48% (95%CI, 41-54%).

**Conclusions:** Vitamin B12 levels were shown to be highly heritable and here we report the first heritability estimation for vitamin B12 levels. The high heritability obtained for vitamin B12 levels is suggesting that further genetic analysis have to be considered in order to identify genetic variants responsible for vitamin B12 variation.

#### P10.86

#### Comparison of VKORC1 haplotype profile and CYP2C9 polymorphisms as determinants of coumarin dose in Hungarian and Roma population samples

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Anticoagulant action of coumarins is mainly moderated by the VKORC1 and CYP2C9 genes. By means of haplotype tagging SNPs (G-1639A, G9041A, C6009T) we characterized Hungarian (n=510) and Roma (n=451) populations for the VKORC1\*1, \*2, \*3, \*4 haplotypes, and for the CYP2C9\*2, CYP2C9\*3 allelic variants. The samples were analyzed by PCR-RFLP assay and direct sequencing. In Hungarians the VKORC1\*1, \*2, \*3, \*4 haplotypes were 3, 39, 37, 21%, by contrast, in the Roma populations were 5, 30, 46, 19%, respectively. Comparing the genotypes of Roma and Hungarian populations difference was found in the \*2\*2 (6.87 vs. 13.5%), \*2\*4 (13.9 vs. 19.2%), 3\*3 (21.9 vs. 13.7%) VKORC1 genotypes. The frequencies of CYP2C9\*1, \*2, \*3 alleles in the Hungarian population were 0.787, 0.125, 0.088 and in Roma 0.727, 0.118, 0.155, respectively. The distribution of \*1\*1, \*1\*2, \*1\*3, \*2\*2, \*2\*3, \*3\*3 genotypes in Hungarians were 0.620, 0.195, 0.139, 0.021, 0.015, 0.011, while in Roma were 0.533, 0.168, 0.219, 0.011, 0.047, 0.022, respectively. Significant difference was found between Hungarian and Roma population considering the CYP2C9\*3 frequency and \*1\*1, \*1\*3, \*2\*3 genotypes ( $p < 0.005$ ). The distribution of low and high dose determining VKORC1 haplotypes and CYP2C9 variant alleles in the Hungarian population is similar to that observed in other European populations. By contrast, the Roma population differs from Hungarians, and from most of other Caucasian groups and from population samples from reported from India in the incidence of VKORC1 haplotypes and CYP2C9 common variants.

**P10.87****Phylogeography of human Y chromosome haplogroup R1b1b2 (R-M269) in Europe**

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The human Y chromosome haplogroup R1b1b2 (R-M269) displays an extremely wide geographic distribution within Europe, with a decreasing frequency cline from Iberia (frequencies up to 90%) towards the Balkans (usually less than 10%). Previous studies have proposed that the observed R1b1b2 frequency cline is due to a population expansion from an Iberian Ice-age refugium after the LGM (Malaspina et al. 1998; Semino et al. 2000).

In this study, we explored the phylogeography of the human Y chromosome haplogroup R1b1b2 by analyzing more than 2,000 males from Europe. The haplogroup-defining marker M269 (Cruciani et al. 2002), and two additional internal markers (U106 and U152, Sims et al 2007) which identify internal branches (R1b1b2g and R1b1b2h) were analyzed. The paragroup R1b1b2\*(xR1b1b2g, R1b1b2h) and the haplogroups R1b1b2g and R1b1b2h showed quite different frequency distribution patterns within Europe, with frequency peaks in the Iberian Peninsula, northern Europe and northern Italy/France, respectively. The overall frequency pattern of R1b1b2 haplogroup is suggestive of multiple events of migration and expansion within Europe rather than a single and uniform spread of people from an Iberian Ice-age refugium.

## References:

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**P10.88****Diversity of Y-STRs in the representative sample of the local human population of Canton Sarajevo residents**

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In one of our previous population studies of B&H human population, we used twelve Y-chromosomal short tandem repeats loci incorporated in the *PowerPlex® Y System* to generate Bosnian Y-STR referent database. Wishing to test these results in order to obtain specific results in various DNA analyses for the local human population of Canton Sarajevo residents, we have decided to test DNA samples collected from 100 unrelated healthy male individuals from Sarajevo at twelve Y-linked short tandem repeats loci. Qiagen Dnaeasy™ Tissue Kit was used for DNA extraction from buccal swabs and *PowerPlex® Y System* (Promega Corp., Madison, WI) has been used to simultaneously amplify by PCR 12 Y-STR loci. The STR loci that were used are: DYS19, DYS385a, DYS385b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438 and DYS439. The total volume of PCR reaction was 5µl. PCR amplifications were carried out in PE GeneAmp PCR System Thermal Cycler. Electrophoresis of the amplification products was preformed on an *ABI PRISM 310* genetic analyzer (ABI, Foster City, CA) according to the manufacturer's recommendations. The raw data were compiled and analyzed using the accessory software: *ABI PRISM®Data Collection Software and Genemapper®* v3.2. In addition, we compared obtained Sarajevo data with the data previously obtained from the entire Bosnian and Herzegovinian population, as well with geographically closer European populations. The results of this study will be used as guidelines in additional improving of investigation of genetic relationship between recent local B&H populations, both isolated and opened, initiated in our previous researches.

**P10.89****The genetic position of Western Brittany (Finistère, France) in the Celtic Y chromosome landscape**

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Brittany, a large peninsula located at the western part of France, is of particular interest because of its historical settlement and its relative geographic and cultural isolation. Brittany was invaded by waves of migration from Britain and Ireland between the 4th and 7th centuries and, therefore, belongs to the Brythonic branch of the Insular Celtic language. We have focused our study on the department of Finistère, the most western territorial unit of Brittany, and its administrative and historical areas. To explore the diversity of the Y-chromosome, we analyzed a total of 348 unrelated males using a combination of 23 biallelic markers and 12 microsatellite loci. The molecular analysis revealed that 82.2% of the Y chromosomes fell into haplogroup R1b, placing Finistère within the Western European landscape. Interestingly, at a microgeographical level, differences were detected by the haplogroup R1a\* being confined to the south of the department, while haplogroups E3b, F, G, J2, K and R1a1 were found in the north. Nevertheless, geographical distribution of haplogroups and haplotypes suggested territorial homogeneity inside Finistère. Most of the Y-chromosomal gene pool in Finistère is shared with European, especially British, populations, thus corroborating the historical reports of ancient migrations to Brittany. Finally, the results are consistent with those obtained from classic genetic markers and support the Celtic paternal heritage of the Finistère population.

**P10.90****The N1b (N2) Y-chromosome haplogroup structure in Khanty gene pool**

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Haplogroup N1b (N2) is one of the major Y-chromosome variant in South Siberia and North Asian Finno-Ugric population. In present study a total sample of 112 males from two villages of Khanty-Mansi Autonomous Area (Kazym and Russkinskie) of Siberia was typed with 52 Y-chromosomal SNP markers. Five haplogroups (N1b (N2), N1c1 (N3a), Q\*, R1a1 and R1b1b2 (R1b3)) were found in the Khanty gene pool. The maximal frequency has N1b (N2) haplogroup: 67% in Kazym population and 41% in Russkinskie population.

An analysis of molecular variance (AMOVA) based on Y-chromosomal haplogroups showed that the variation observed between the two populations is 20.6%, indicated the high genetic subdivision of Khanty gene pool. The STR genetic diversity was H=0.49 in Kazym population and H=0.68 in Russkinskie population.

To reconstruct the structure of Y-chromosome haplogroup N1b (N2) in Khanty we have analyzed the diversity of seventeen Y-chromosomal microsatellite (STR) loci. Median network analysis of STR data demonstrates that haplogroup N1b (N2) is represented by two subclusters, showing recent expansion times. It is also shown that haplogroup N1b (N2) is characterized by high frequency of founder haplotype, which includes 40% of all explored Y-chromosomes. The N1b founder haplotype in Khanty is the same as the ancestral haplotype for the whole lineage; and Khanty represents the maximum of the frequency suggesting the North Siberian origin of N1b.

**P11. Genomics, Genomic technology and Epigenetics****P11.001****Simultaneous mutation and CNV detection by multiplex PCR based GS FLX sequencing**

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We evaluated multiplex PCR amplification as a front-end for high-throughput sequencing to widen the applicability of massive parallel sequencers for the detailed analysis of complex genomes. Using multiplex PCR reactions, we sequenced the complete coding regions of 7 genes implicated in peripheral neuropathies in 40 individuals on a Genome Sequencer FLX. The resulting dataset showed highly specific and uniform amplification. Comparison of the 454 data with the dataset generated by Sanger sequencing confirmed the detection of all

variants present and proved the sensitivity of the method for mutation detection. In addition, we showed that we could exploit the multiplexed PCR amplicons to determine individual Copy Number Variation (CNV), increasing the spectrum of detected variations to both genetic and genomic variants. We conclude that our straightforward procedure substantially expands the applicability of the massive parallel sequencers for sequencing projects of a moderate number of amplicons (50 - 500) with typical applications in resequencing exons in positional or functional candidate regions and molecular genetic diagnostics. Completely in line with this conclusion, we are now developing assays for the CFTR and BRCA1/BRCA2 coding sequences.

#### P11.002

#### Promoter polymorphism -368 C/T of the acetyl-CoA carboxylase 2 gene ACACB influences activity and nuclear protein binding in HepG2 cells

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Acetyl-CoA carboxylase 2 (ACC2) catalyses the formation of malonyl-CoA, a key regulator of fatty acid oxidation. Increased ACC2 activity would reduce fatty acid oxidation because malonyl-CoA inhibits entry into mitochondria via carnitine palmitoyltransferase I (CPTI). The transcriptional regulation of the ACC2 gene (ACACB) is complex and involves many factors which may be tissue-specific. In liver, it has been established that promoter P-II controls transcription of the ACACB gene and sterol regulatory element-binding protein-1 (SREBP-1) regulates expression. We studied the functional impact of a single nucleotide polymorphism (SNP) in the promoter region -368 C/T (rs16939972) in HepG2 cells in the presence of SREBP-1a. The -368 C/T SNP was selected as the closest to known SRE sites, validated on dbSNP and occurring at a frequency of over 0.05 in the CEPH population (MAF=0.25). The promoter construct carrying the -368 T allele showed 1.35 fold lower activity than the construct carrying the -368 C allele. Electrophoretic mobility shift assays (EMSA) revealed the -368 T allele has a higher affinity for nuclear proteins than the -368 C allele, suggesting the -368 C/T SNP may bind to a repressor. EMSA competition experiments using unlabelled oligonucleotides containing transcription factor binding motifs, showed that the -368 C/T SNP may bind to C-Myb, GATA and/or glucocorticoid receptor (GR) proteins. In conclusion, the data suggest the ACACB -368 C/T polymorphism is a regulatory SNP that affects promoter activity in HepG2 cells in the presence of SREBP-1a and alters nuclear protein binding affinity.

#### P11.003

#### Molecular and cytogenetic characterisation of human albumin transgenic goat fibroblasts as a source of nuclei in the somatic cloning

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The production of pharmaceutically important human proteins in the mammary gland of transgenic animals constitutes an important field of biotechnology.

In this study goats' fibroblasts were transfected by lipofection with transgene, which contained human gene encoding albumin under the control of the tissue specific WAP promoter. Transfected fibroblasts were cultured with selective medium with blasticidin.

Transgene integration was examined by PCR method. Chromosomal aberrations were examined using the GTG-binding pattern. Fluorescence in situ hybridization (FISH) enabled the mapping of transgene specific DNA sequences.

Transgenic cells are going to be used as a source of nuclei in the experiments of obtaining transgenic goats by somatic cloning technique. The application of tissue specific WAP promoter allows to reduce the expression of the transgene to mammary gland. Human albumin is going to be found only in the milk of animals being accurately in the period of lactation. After the separation of albumin from milk components it would be applied in medical treatment.

#### P11.004

#### Rare Allele Enrichment by Snapback Primer

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Allele specific PCR for selective amplification of minority alleles is widely employed in detecting cancer mutations. Allele specific PCR using Snapback primers is a closed-tube intramolecular method that does not require fluorescently labeled probes. Only two PCR primers are required, with the addition of a short-tail of nucleotides on one primer that results in an intramolecular hybridization probe. Genotyping is performed by high resolution melting of the hairpin. Rapid cycle PCR enables selective amplification of minority alleles by lowering the extension time to 0 second. Using a carousel LightCycler® or LS-32 (Idaho Technology), this requires less than 25 minutes. High-resolution melting is then performed on an HR-1 or LS-32. The detection sensitivity of point variants is up to 1 in 1000. A single hotspot mutation of the BRAF gene (1799 T>A, V600E) was analyzed in thyroid tumor tissue and needle samples from 45 genotyped patients. It is the most common change in papillary thyroid carcinoma resulting in more than 80% of thyroid cancers. The sensitivity and specificity were 100% compared to histology and other molecular methods. An EGFR exon19 in-frame deletion was used for small deletion detection; a 1:10000 deletion mutations to wild type ratio could be analyzed. Somatically acquired mutations in the EGFR gene in non-small cell lung cancer are associated with a significant clinical response to tyrosine kinase inhibitors. Enriching minority alleles with Snapback primers is attractive because only PCR reagents and dsDNA dye are needed. No expensive modified oligonucleotides, separations, purification or addition steps are necessary.

#### P11.005

#### Impaired activity of serum alpha 1-antitrypsin in carriers of p.G320R variant

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The alpha 1-antitrypsin (A1AT) gene is highly polymorphic, with more than 100 genetic variants identified so far. Some of these variants can affect A1AT protein concentration and/or function and lead to pulmonary and/or liver disease. This study reports on the characterization of p.G320R variant found in two patients - one with emphysema and other with lung cancer. This variant results from a single base-pair substitution in exon 4 of A1AT gene (Gly-320[GGG]→Arg-320[AGG]) and has been characterized as P by isoelectric focusing. Functional evaluation of A1AT p.G320R variant was performed by determination of specific trypsin inhibitory activity in two patients with pulmonary disorders, carriers of p.G320R variant, and 19 healthy individuals, carriers of normal A1AT M variants (M1, M2, M3, M1M2 and M1M3). Results showed that specific trypsin inhibitory activity was lower in both emphysema and lung cancer patients (2.45 mU/g and 2.07 mU/g respectively) in comparison with values obtained in carriers of normal A1AT M variants (range 2.51 - 3.71 mU/g). This A1AT variant is associated with reduced functional activity of A1AT protein. Considering that it was found in patients with severe pulmonary disorders, this variant might be of clinical significance. In order to completely characterize this variant and estimate whether it is associated with pulmonary disorders, expression concentration, secretion rate and tendency to aggregate should also be analyzed.

#### P11.006

#### The hunt for *de novo* chromosomal aberrations in patients with MR/MCA

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Array-based comparative genomic hybridization has become an indispensable tool in the hunt for small *de novo* chromosomal aberrations that are presumed to be present in patients with multiple congenital

abnormalities and/or idiopathic mental retardation. The discovery of neutral copy number variants has complicated these analyses. There is thus a need for procedures that efficiently distinguish inherited from *de novo* aberrations. We have implemented an oligo-based array platform for the postnatal screening of patients with MR/MCA for cryptic microdeletions and duplications in addition to karyotyping. One hundred patients have been analysed simultaneously with their parents. On one array the patient is hybridized to a reference sample constituted of a pool of either 40 males or females. On a second array, the parents are hybridized with opposite dyes. Separate ZlogR files for the parents are subsequently generated using home made software package that creates export files can be uploaded into several commercial data analysis platforms. In this procedure ~89% of all aberrations detected by the patient could directly be traced back to either of the parents. This approach constitutes a cost-efficient and fast way to determine the *de novo* nature of the aberrations that are seen in the patient. Furthermore, we have used the data for 400 healthy parents to generate a database of local CNVs. This database has proven to be of high value in the analysis of individual patients. Results on the analysis of 100 trios and 600 additional patients will be presented.

#### P11.007

#### Optimisation and standardisation of sample preparation with the Bead-beating technology in genomics research

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In the context of sample preparation and cell lysis, Bertin Technologies (France) has developed a technology dedicated to the homogenization and grinding of soft to hard materials. The goal is to improve the first critical step in any molecular biology process and follow the latest requirements of analysis equipments which have radically improved in terms of throughput, reproducibility, detection limits and linearity. Following specific mechanical engineering studies of bead beating technology, a high speed figure-8 multidirectional motion gives shaking energy to the beads that grind/homogenize samples in sealed tubes. This patented solution Precellys24 plays a large part in the analyse chain of rapid method to extract and detect or quantify DNA, RNA or proteins. Thanks to Cryolys option, temperature inside Precellys24 tubes is maintained at an optimal level during homogenization. Cryolys technology permits temperature-sensitive molecules to keep their native state for any analysis.

Bertin and its partners have been investigating mechanical lysis with the Precellys bead beater vs. manual, chemical or sonicator methods. Several applications on protein and RNA extraction from human or animal tissues illustrate the contribution of this equipment to the improvement of genomics research.

Bead beating technology was successfully evaluated in these applications and satisfied users in term of efficiency without degradation of the material, reproducibility, time and labour saving that are mains items to consider.

#### P11.008

#### DAME and RGG - Two software tools from the AIT bioinformatics software lab

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High throughput methods, such as microarrays or next generation sequencing, are widely used in molecular research. Target annotation, sequence analysis, signal analysis and further experimental planning increasingly require the combination of multiple bioinformatics resources.

DAME (Data Analysis Management and Exploration) is a user-friendly software for high throughput analysis of DNA, RNA and protein sequences, feature selection and assay design. The entire analysis data flow is organized into a virtual table, thus batch processing of thousands of sequences can be performed very efficiently. Function usage is unified by a standardized IPOP (input/parameter/output panel).

RGG (R GUI Generator) is a general GUI framework for R scripts that has the potential to introduce R statistics (R packages, built-in functions and scripts) to users with limited programming skills and helps to bridge the gap between R developers and GUI-dependent users. RGG

aims to abstract the GUI development from individual GUI toolkits by using an XML-based GUI definition language. GUIs are generated in runtime from defined GUI tags that are embedded into the R script. The RGG project further includes the development of a web-based repository for RGG-GUIs. RGG is an open source project and can be downloaded freely at <http://rgg.r-forge.r-project.org>

#### P11.009

#### NeuroMiner: Web-based platform for integrated microarray analysis

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Global gene expression studies have provided new insights into the pathogenesis of neurodegenerative diseases. A hallmark of the scientific process is the reproducibility of published outcomes, and yet comparing the results of microarray studies has proven difficult. Although experimental results of global gene expression measuring may identify common genes, each research group will typically produce different list of statistically significant differentially expressed genes, which calls into question the reliability and validity of each gene list. There are two general approaches to integrating microarray studies: meta-analysis of the primary data by merging data from multiple studies, and comparative analysis of the published results (i.e., gene lists). The application of conventional meta-analysis to raw microarray data is complicated by differences in the type of microarray used, gene nomenclatures, species, and analytical methods. An alternative approach to combining multiple microarray studies is to compare the published gene lists. However, manual combining data from published gene lists is often a very tedious and time-consuming task. In order to address these issues, we have developed a web-based platform, called NeuroMiner to house and integrate the results of published microarray experiments from selected neurodegenerative diseases (Alzheimer's disease, Huntington's disease, Multiple sclerosis, and Down syndrom). NeuroMiner allows researchers to compare the results of similar studies in order to identify consistent expression patterns, as well as helping experimenters to compare their own data to published microarray results. NeuroMiner is available free of charge for academic purposes at <http://www2.arnes.si/~akastr1/>.

#### P11.010

#### TaqMan® Arrays for pathway based biomarker discovery

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Molecular biomarker discovery usually results in the identification of a single gene or small number of genes that can be used to diagnose a disease or predict a therapeutic outcome. Intensive genome-wide transcriptome studies are commonly used to generate the initial list of genes which display differential expression across treated versus untreated tissues, or diseased versus normal tissues. Recently, attention has been focused on identifying pathway based biomarkers which involves direct screening of genes from a specific pathway or a set of biologically or functionally related genes (gene signatures). To this end we have developed >100 TaqMan® Gene Signature Arrays for human, mouse and rat in 384-well micro-fluidic card and 96-well plate format. TaqMan Arrays allow reproducible and quantitative real-time, high-through put screening across many samples and genes and provide higher sensitivity, specificity and dynamic range than DNA microarrays. Genes for each signature panel were selected from the GeneAssist™ Pathway Atlas ([http://www4.appliedbiosystems.com/tools/pathway/all\\_pathway\\_list.php](http://www4.appliedbiosystems.com/tools/pathway/all_pathway_list.php)), external collaborations, and/or literature curation. Here we present results from the development of a Lipidomics Gene Signature Array for mouse. Data from the mouse 96-well array format comparing expression of lipid genes in primary microglia and primary bone-derived macrophage after treatment with bioactive compounds will be presented.

#### P11.011

#### The Folliculin (FLCN) mutation database: an online resource for FLCN sequence variants involved in Birt-Hogg-Dube syndrome

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Birt-Hogg-Dubé (BHD) syndrome is an autosomal dominantly inherited familial cancer syndrome characterised most commonly by the development of facial fibrofolliculomas, pulmonary cysts (predisposing to spontaneous pneumothorax) and renal tumours. Germline mutations in FLCN on 17p11.2 have been reported in patients with BHD and also in patients with isolated primary spontaneous pneumothorax. The function of the FLCN gene product, folliculin, is not well characterised but recent studies have suggested that it may be implicated in the regulation of several key signalling pathways including the AMPK-mTOR route.

We describe the FLCN mutation database which is based on the Leiden Open (source) Variant Database (LOVD) system. To date the variants described in the database were extracted from the published literature and from unpublished mutations detected in Birmingham, UK. However the database will be expanded to include further mutations from partners in the European BHD Consortium (<http://www.europeanbhdconsortium.eu/members.aspx>). Researchers can also directly submit new sequence variants (to [Derek.Lim@bwhct.nhs.uk](mailto:Derek.Lim@bwhct.nhs.uk) or [E.R.Maher@bham.ac.uk](mailto:E.R.Maher@bham.ac.uk)). The FLCN mutation database offers a valuable resource and tool for clinicians involved in the management of BHD patients, clinical geneticists and researchers.

#### P11.012

#### Grid-enabling G2P association studies: a knowledge discovery scenario

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The heterogeneity and scale of the data generated by high throughput (HTP) genetic association studies calls for the seamless access to respective distributed data sources. In this context, GEN2PHEN devotes efforts on the utilisation and harmonisation of *Semantic Grid* (SG), *Web Services* (WS), *Scientific Workflows* (SWf), and *Knowledge Discovery* (KD) technology. The task is realised in a Grid-enabled G2P SWf (GG2P).

GG2P SWf unfolds into five steps: (i) using EBI's and custom-made WSs registered genotype experiments are accessed from public repositories (e.g., ArrayExpress) - respective XML files are downloaded, (ii) custom-made WSs are called to parse the XML files and download the respective raw data, (iii) with custom-made WSs the data are brought into formats suitable for data-analysis; (iv) data-mining algorithms, wrapped as WSs, are called to discover indicative SNPs that discriminate between phenotypic classes, and (v) special WSs are called to map the discovered most-discriminант SNPs to corresponding genome regions, and visualise them within the Ensembl genome browser.

GG2P SWf was applied on a HTP SNP-genotyping experiment that concerns 36 BRCA and 36 control/normal samples (E-GEOID-3743). We were able to identify a set of about 100 SNPs, the heterozygosity profile of which exhibit a clear LOH in the BRCA cases.

GG2P utilises a BPEL-compliant Wf editing and enactment environment. We plan to devise an integrated Grid reference architecture for the GEN2PHEN environment able to support GG2P-like scenarios and SWs.

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#### P11.013

#### High-density, flexible arrays for genome-wide or targeted analysis of epigenetic mechanisms of disease

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Epigenetic mechanisms, such as DNA methylation and histone modification, play critical roles in the development of many human diseases including cancer, paediatric syndromes and genetics disorders. Under-

standing the role epigenetics plays in the development of disease will ultimately lead to the development of diagnostics and hopefully preventative and therapeutic options. Roche NimbleGen's highly flexible, high density HD2 microarray platform, with 2.1 million (2.1M) long-oligonucleotides probes per array, affords researchers the opportunity to examine epigenetic events using ChIP-chip and MeDIP-chip assays at unprecedented scale and resolution. We will demonstrate (1) the comprehensive, sensitive, reproducible DNA methylation analysis possible using MeDIP and the 2.1M DNA Methylation arrays, (2) the utility of the positive, negative and non-CG controls regions present on these arrays in analyzing MeDIP experimental performance, and (3) the power of combining MeDIP, sequence capture, and bisulphite sequencing to analyze DNA methylation at single-base resolution across the genome.

#### P11.014

#### Amplification of intermethylated sites, Bioinformatics and Capillary electrophoresis: the ABC of the cancer methylomes

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Amplification of intermethylated sites (AIMS) is the method that best fits the requirements to make it a universal unbiased differential methylation screening approach. Still it is not frequently elaborated because it possesses drawbacks in fragments resolution and mapping of the identified differentially methylated loci. AIMS generates significant numbers of PCR products, thus the mode of DNA fragments detection has to be optimized to achieve appropriate resolution and easy mapping to the genome. We have achieved single-nucleotide resolution by capillary electrophoresis (CE). At the same time CE does not generally provide preparative option, thus sequencing the bands in order to identify the genomic locations of the fragments has to be substituted by another approach. Knowledge of exact nucleotide length of a predicted or practically obtained AIMS product allows its *in silico* identification in genomic context. In order to utilize this option we have designed specific software, AIMS *in silico*, which predicts all possible outcomes of AIMS and labels the fragments of certain lengths with unique sequence descriptors allowing their genomic positioning. Elaboration of this AIMS-bioinformatics-CE approach allows rapid characterization of normal and cancer methylomes, assessment of tissue-specific methylation, identification of novel genes prone to abnormal methylation in cancer, and rough evaluation of the cancer methylome landscapes for different types of disease. We present the results obtained on paired (tumor/control) breast tissue samples, control peripheral blood samples and extraembryonic tissues in order to demonstrate the validity and potentials of our approach, and describe novel cancer related genes identified in breast cancer.

#### P11.015

#### Expression of Centa2 and Suz12 during mammalian heart development

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Cardiovascular malformations (CVMs) have a higher incidence in patients with NF1 microdeletion syndrome, compared to classical NF1 patients, presumably owing to haploinsufficiency of the genes lying in the deletion interval. Searching for CVMs candidate genes inside the deletion, we focused our attention on three genes, CENTA2, SUZ12 AND UTP6. Whole mount *in situ* hybridization (WISH) on mouse embryos showed high expression of Centa2 in heart at 9-10 dpc, and of Suz12 in the atrium around 10 dpc, suggesting their involvement in heart development and CVMs onset. RT-PCR analysis on zebrafish embryos showed Centa2 and Suz12 expression in oocytes and throughout all the analyzed stages (8 cells-120 hpf). Centa2, but not Suz12 was also present in adult heart. We thus carried out WISH experiments on zebrafish embryos to characterize Centa2/Suz12 spatio-temporal expression profiles. At 24 hpf we observed a diffuse Centa2 specific hybridization signal in the cephalic and medial portion of the embryo, which becomes stronger starting from 48 hpf. At 48-72 hpf

Centa2 is also expressed in liver and pectoral fin bud. We observed a weak signal in the bulbus arteriosus at 48-72 hpf, but not in the remaining heart portions. Suz12 shows an intense hybridization signal in the medial and rostral region of the embryo at 30-72 hpf, but no expression in the heart. Considering that zebrafish heart has two chambers, we speculate that Centa2 and Suz12 might be important for cardiac morphogenesis in the most evolved organisms, such as mammals, which have a four-chambered heart.

#### P11.016

#### The regulation of CDK5R1 gene expression by miRNAs may have a role in Alzheimer's disease

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CDK5R1 encodes for p35, an activator of CDK5, which is involved in neuronal migration and differentiation during CNS development and is hyperactivated in Alzheimer's disease (AD) leading to Tau hyperphosphorylation. We recently reported that the large 3'UTR of CDK5R1 contains regulatory elements affecting transcript stability. Many microRNAs (miRNAs) target sites have been predicted by PicTar software. We evaluated the expression of nine pre-miRNAs, among the 20 miRNAs predicted to bind CDK5R1, in six cell lines. Among the expressed miRNAs, we observed that five of them present a high number of target sites with a free energy <-20 kcal/mol. A Real-Time PCR of the above miRNAs showed an inverse correlation between miR-107/miR-103 levels and p35 expression, suggesting a negative effect of the two miRNAs on CDK5R1 expression. We overexpressed miR-107 by transfecting the specific precursor in neuroblastoma SK-N-BE cells and observed a 75% reduction in p35 expression, while the transfection of anti-miR-107 led to a 2.3 times increase of p35 in comparison to the control. The obtained findings indicate that miR-107 regulates CDK5R1/p35 expression. It's worth to be noted that under-expression of miR-107 has been implicated in the acceleration of AD. Experiments on the other miRNAs of interest are in progress. Luciferase constructs will be used to validate the predicted miRNA target sites in CDK5R1 3'UTR. Our findings on CDK5R1 regulation by miRNAs allow us to hypothesize that a new pathogenetic miRNA-mediated mechanism might influence the CDK5 phosphorylation activity on Tau, leading to AD progression.

#### P11.017

#### Searching for centaurin- $\alpha$ 2 interacting proteins: evidence of interaction with tubulin- $\beta$

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Centaurin- $\alpha$ 2 belongs to the centaurin family and is characterized by a zinc binding domain similar to Arf-GAP and by two PH domains. Its expression pattern and function have not yet been extensively investigated. Centaurin- $\alpha$ 2 was shown to bind PIP2 and PIP3 and to localize at plasma membrane, promoting the release of GTP and the consequent inactivation of Arf-6, a protein involved in the regulation of intracellular vesicular trafficking and in cytoskeletal rearrangement. We recently studied the expression profile of CENTA2 mRNA by in situ hybridization, during mouse embryo development and we found it is expressed in early developmental stages of encephalon and heart.

With the final aim of elucidating the centaurin- $\alpha$ 2 molecular pathways and its biological role/s, we searched for interactors by means of the yeast two-hybrid assay.

An interaction with the C-terminal region of tubulin- $\beta$  has been observed and confirmed by co-immunoprecipitation. This portion of tubulin- $\beta$  is involved in the binding of MAPs and motor proteins. Considering that tubulin- $\beta$  was found to bind phospholipase Cy1 through its PH domains, we hypothesized an interaction between centaurin- $\alpha$ 2 mediated by this domain.

The yeast two-hybrid assay allowed us also to detect an interaction with nucleoporin NUP53, a component of the nuclear pore complex, that will be confirmed by co-immunoprecipitation.

According to our evidence, that will be further investigated, we propose that centaurin- $\alpha$ 2 can be localized at the plasma membrane, the

cytoplasm and the perinuclear region, and can translocate through microtubules anchoring, according to its hypothesized role in vesicular trafficking.

#### P11.018

#### Detection of CFTR copy-number variations in Cystic Fibrosis and Congenital Bilateral Aplasia of the Vas Deferens patients by custom array CGH

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Introduction : Mutation in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene is responsible of a large spectrum of clinical phenotype from severe CF to male sterility due to Congenital Bilateral Aplasia of the Vas Deferens (CBAVD). More than 1500 CFTR mutations have already been described and new semi quantitative approaches have been developed to evidence gross rearrangements in the 27 exons of the gene. These deletions account for about 1-3 % of the CF disease. We have described 8 new rearrangements and characterized their breakpoint junctions. However this technique missed the intronic region as well as the 5' and the 3' UTR part of the gene.

Methods : We designed a custom Agilent 15K array on the locus CFTR. This oligonucleotide array enable us to cover 2 Mb encompassing the CFTR locus as well as the gene itself. We analysed positives and unaffected controls, 109 CF and 160 CBAVD patients with an incomplete genotype.

Results and conclusion: We evidenced the 11 deletions collected in our laboratory as controls. We also identified 5 duplications and 2 deletions in the CFTR gene. About the CBAVD patients, we observed 2 intronic deletions. This custom array allow a rapid design of primers to amplify the 5' and 3' breakpoints junctions of the variation and to obtain the sequence permitting the characterization of the defect at the molecular level. Our array is an excellent tool allowing a rapid detection of the rearrangements located throughout the 200 kb genomic region of the CFTR gene.

#### P11.019

#### SNP-based system improves the applicability of qPCR for chimerism monitoring

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Bone marrow transplantation (BMT) is established medical procedure used to treat various malignant and nonmalignant hematological diseases. The key test used to predict disease relapse and graft rejection is monitoring of post-transplant chimerism.

The basic principle in the detection of chimerism is the utilization of differences between recipient and donor genomes. Single nucleotide polymorphism is the most abundant form of genetic variability in the human genome. SNPs have proved to be particularly useful as markers for monitoring chimerism after BMT, because they are stable and unique and can be analyzed by sensitive qPCR. However, before it becomes established method for routine chimerism monitoring, qPCR marker set for every transplant pair should be available.

The aim of our study was to design and validate a new SNP allele-specific system to supplement already existing InDel primer panel. We present an approach for the economic in-house design of SNP allele-specific qPCR primers/probe sets with locus individualized reference system that allows accurate quantification of respective informative locus using simple ddCt method without standard curves.

We designed ARMS-primers/probe sets specific for seven biallelic SNP loci and validated them in a population of 30 transplant pairs. Detection limit of our system was 0.1%. Combination of our SNP-qPCR system and InDel primers increased recipient genotype identification from 86.6% (113/127) to 96.6% (123/127) when tested in a population of all transplant pairs. The developed SNP marker panel may contribute to the successful screening for discriminative markers and further extend the overall number of SNPs available for chimerism evaluation.

**P11.020****Long range expression effects of copy number variation: insights from Smith-Magenis and Potocki-Lupski syndrome mouse models**

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To study the effect of structural changes we assessed gene expression in genomic disorder mouse models. A microdeletion and its reciprocal microduplication, which model the rearrangements present in Smith-Magenis (SMS) and Potocki-Lupski (PTLS) syndromes, respectively, have been engineered. They show phenotypic features similar to those identified in human patients. We profiled the transcriptome of five different tissues affected in patients in mice with 1n (Deletion/+), 2n (+/+), 3n (Duplication/+) and uniallelic 2n (Deletion/Duplication) copies of the same region in an identical genetic background.

The most differentially expressed transcripts were ranked. A highly significant propensity, are mapping to the engineered SMS/PTLS interval. A statistically significant overrepresentation of the genes mapping to the flanks of the engineered interval was also found in the top-ranked differentially expressed genes. A phenomenon efficient across multiple cell lineages and that extends along the entire chromosome, megabases from the breakpoints. These long-range effects are unidirectional and uncoupled from the number of copies of the CNV genes. Our results suggest that the assortment of genes mapping to a chromosome is not random. They also indicate that a structural change at a given position may cause the same perturbation in pathways regardless of gene dosage. An issue that should be considered in appreciating the contribution of this class of variation to phenotypic features.

We will also discuss the molecular networks that are altered in the different models. This network analysis enables the identification of metabolic pathways that potentially play a function in the SMS/PTLS phenotypes.

**P11.021****Assessment of Copy Number Variation on a Large Population-Based Cohort, The Rotterdam Study**

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**Background:** Copy number variants (CNVs) are a form of genetic variation where individuals have amplifications or deletions (>1kb) in different regions of the genome. They occur commonly in the human genome, often affecting genes and potentially influencing human traits.

**Aim:** Assess the prevalence of CNVs at the population level and their association with multiple traits and diseases.

**Methods:** We obtained normalized intensity data of 5,824 individuals of the Rotterdam Study (RS), a population-based cohort of elderly men and women of Dutch origin, genotyped on the Illumina 550K array and applied two computational methods that use intensity signals (QuantisNP) and deviations from Hardy-Weinberg equilibrium and information on LD patterns (Trityper) to detect both common and rare CNVs. **Results:** After quality control, Trityper identified 775 sites with evidence of a common deletion, 400 of which had a frequency > 0.05. QuantisNP identified 49,229 events (mapping to 26,162 genomic locations) with an estimated false positive rate of 1 / 100,000. We performed a case-control association study in 5,287 participants of which 809 had clinical evidence of osteoporotic fractures. A 210 kb deletion located on the 6p25 chromosomal region was found present in seven individuals of the population (0.1%). The CNV is in the vicinity of the CYDL gene and was found significantly associated with the risk of osteoporotic fracture OR:32 ([95%CI 3.4-262]; p=3x10-7; p = 0.02 after correction for multiple testing).

**Conclusions:** CNV association analyses for multiple cardiovascular, neurological, locomotor and ophthalmological disease traits and conditions is currently underway.

**P11.022****Copy Number Variation Analysis Using Quantitative TaqMan® Copy Number Assays**

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Recent whole-genome studies have identified 6225 Copy Number Variant (CNV) loci, which are large-sized deletion or duplication events. CNV regions can influence gene activity and certain CNVs have been associated with disease susceptibility. Copy number changes are also detected in microdeletion/microduplication syndromes that are associated with genomic disorders. Although array-based technologies are powerful for large-scale CNV and microdeletion/microduplication discoveries, more quantitative technologies with high accuracy, specificity and sample throughput are necessary to both validate microarray-identified copy number changes and to examine such changes in large samples sets. To meet these needs, Applied Biosystems has developed TaqMan® Copy Number Assays. Using a proprietary design pipeline, assays have been generated to high quality, genome-wide targets. TaqMan Copy Number Assays are run with a reference assay, known to be present in two copies in a diploid genome, and gDNA in a duplex real-time PCR. Sample copy number is determined by relative quantitation analysis using Applied Biosystem's CopyCaller™ Software tool. Here we show data generated from assays targeting X chromosome, CNV-associated OMIM genes, and chromosomal regions associated with genomic disorders. The assays were tested with different genomic DNAs including HapMap samples and samples with known deletions/duplications. These data sets demonstrate excellent TaqMan Copy Number Assay performance with high accuracy and specificity. We also applied TaqMan Copy Number Assays to array CGH validation and demonstrated concordance between platforms.

**P11.023****Deep surveying of whole transcriptome under CNV effect**

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Copy number variation (CNV) of DNA segments has recently been identified as a major source of genetic diversity, but a comprehensive understanding of the phenotypic effect of these structural variations is only beginning to emerge. My host laboratory has generated an extensive map of CNV in wild mice and inbred strains. These variable regions cover ~11% of their autosomal genome. Tissue transcriptome data show that expression levels of genes within CNVs tend to correlate with copy number changes and that CNVs influence the expression of flanking genes. Genes within CNVs show lower expression levels and more specific spatial expression patterns than genes mapping elsewhere. These analyses reveal differential constraint on CNV genes expressed in different tissues. Dosage alterations of brain-expressed genes are less frequent than those of other genes. This study suggests that CNVs shape tissue transcriptomes on a global scale and thus represent a substantial source for within-species phenotypic variation. To unravel the effects of CNV on expression of both coding and non-coding RNA at the nucleotide rather than locus level I propose to use RNA-seq to monitor expression changes of transcripts that map to CNV regions and their flanks. Considering the multiple tissues we plan to investigate and the sequencing coverage we plan to achieve this work should (i) give an unprecedent global and precise view of the mouse transcriptome; (ii) produce the first transcriptome comparison of normal individuals of the population at the nucleotide level; and (iii) help gauge the influence of CNVs on the transcriptome.

**P11.024****Indexed paired-end next-generation sequencing for medical resequencing demonstrated in patients with congenital hyperinsulinism (CHI)**

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Capillary sequencing of the coding region of the ABCC8 and KCNJ11 genes in patients with diffuse Congenital Hyperinsulinism of Infancy (CHI) results in a low mutation detection rate. In 23 out of 44 patients no mutations were found and 9 patients had a heterozygous mutation.

In order to detect further rare variants, we resequenced the entire genomic region of the ABCC8 and KCNJ11 genes (100 kb) on a GA II system in 24 samples and evaluated the sensitivity and specificity of variant detection. The region was amplified with 19 long-range PCR reactions. For library construction, a beta-version of the Illumina multiplexing oligonucleotide paired-end kit was used and up to 12 samples were processed in a single lane. Data were analyzed using the MAQ software. Coverage was between 300- and 5000-fold. Preliminary analysis revealed several false positive variants that were only found on either the forward or reverse strand and showed preference for specific neighboring nucleotides. After filtering for this systematic error, we detected 399 out of 402 previously sequenced coding SNPs/mutations. One of the three differing positions could be accounted for by low coverage in this region. The other two were unambiguous calls. Additionally to the known variants, we detected 88 new SNPs and 8 new indels in the intron- and intergenic regions. 59.1% SNPs and 62.5% indels were confined to a single patient. The other variants are encountered in at least two individuals. Population frequencies of these variants are being investigated.

#### P11.025

##### Finding Copy Number Polymorphism in the Swiss Population

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Having technologies like microarrays and ultra-high throughput sequencing, facilitate the identification of genetic structural variations. SNPs are used to investigate susceptibility to common diseases, yet they explain but a small fraction of the phenotypic variance. Copy number variation (CNV) is the most frequent structural variation in the human genome and encompasses more nucleotides than SNPs. It is likely that CNVs explain at least some of phenotypic variance that cannot be attributed to SNPs, but the extent of this contribution remains unknown.

We present an approach to detect CNVs from Affymetrix arrays and to combine these into Copy Number Polymorphic regions (CNPs). Using an HMM we attribute a copy number state to each SNP probed for a collection of individuals. We then perform a principal component analysis on a window of SNP data across individuals. Only components that explain most of the variance are used to cluster SNPs into CNPs.

Based on this merging method, we report a comprehensive variation map on the CoLaus dataset (Cohort Lausannoise - a 6000 individual population). This map describes 1853 common events (>=1% frequency) and 5797 rare events (frequency between 0.1% and 1%) having respectively a median (mean) size of 140 (201) Kb and 81 (127) Kb. 17.1% of the rare CNVs and 17.97% of the common CNPs were already known. The remaining predicted variants are good candidates for being structural variations in European populations that should be replicated by independent studies. Our CNV map will facilitate association studies of clinical phenotypes with these variants.

#### P11.026

##### Detection of copy number variations (CNV) in patients with mental retardation using high density SNP microarrays

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Chromosomal abnormalities are a major cause of mental retardation. Whole-genome array-based technologies have increased the detection rate of cryptic aneuploidies among these patients up to 10-20%.

DNAs from 109 children with MR and normal G-banded chromosomes and 1 mentally retarded child with a de novo balanced reciprocal translocation were evaluated for rearrangements by Illumina Human610-Quadv1\_B arrays. Data quality was assessed with standard deviation (mean SD: 0.19) and mean absolute deviation (mean MAD: 0.12) of the log<sub>2</sub> intensity ratios. In male samples, a signal-to-noise ratio (mean SNR: 3.82) was calculated. CNVs were called using circular binary segmentation (DNAcopy). Candidate CNVs were compared with known polymorphisms and relevant regions were confirmed by qPCR.

A total of 3,087 CNVs (2,436 losses, 651 gains) ranging in length from 118 to 13,386,172 base pairs (mean 85,224, median 24,331) were detected. 152 out of 3,087 candidate regions were investigated. Of these, 58 (38.2%) were confirmed, 94 (61.8%) were false-positive. 75 (79.8%) of the false positive were detected in regions defined by <10 SNPs, 16 (17%) were indicated by 10-20 SNPs and 3 (3.2%) by >20 SNP.

This study led to the identification of 13 *de novo* CNVs and one maternally inherited Xq13.1 deletion in a male patient. The inheritance of 5 CNVs could not be established because of missing parental DNA.

ID	Chromosome	Gain/Loss	Position UCSC hg18	Number of SNPs	Number of Genes	Length (Mb)	Known Syndrome
28181	4q23.8-q31.1	Loss	136,619,480-140,515,101	770	7	3.895	no
28181	6q16.2-q21	Loss	98,465,339-111,851,511	2628	>50	13.386	no
28181	6q24.1	Loss	141,216,188-142,860,121	212	3	1.643	no
35858	2p12-p13.3	Loss	72,397,638-77,632,757	1074	>50	5.235	no
35929	13q32.3	Loss	98,706,939-98,709,985	6	1	0.003	no
37497	14q11.2	Loss	20,388,473-21,164,794	222	21	0.776	no
38749	16p11.2	Loss	28,363,967-28,549,743	34	8	0.185	16p11.2-p12.2 deletion
38749	16p11.2	Loss	28,733,550-29,283,628	89	9	0.550	16p11.2-p12.2 deletion
39753	6p25.1	Loss	5,104,063-5,406,969	92	2	0.302	no
39753	2p23.1	Gain	29,043,064-29,392,222	60	1	0.349	no
40633	13q12.11	Gain	19,165,733-19,942,459	200	7	0.776	no
43308	17q11.2	Loss	26,024,127-27,392,540	211	14	1.368	1NF1 type I
44289	2q23.3-q24.2	Loss	153,522,479-161,822,306	1617	25	8.229	no
44399	2q13.1	Loss	69,341,389-69,381,997	6	2	0.040	no
33361	4p16.3	Loss*	7,764-1,504,781	289	24	1.497	no
33361	4p16.3-p15.33	Gain*	1,504,782-13,259,183	2991	>50	13.251	no
32608	6q14.1	Loss*	79,576,561-80,104,580	75	3	0.528	no
30921	2p25.3	Gain*	1,069,320-1,752,354	195	3	0.683	no
31166	23p22.2	Gain*	15,952,583-16,694,026	90	4	0.741	no

#### P11.027

##### A genome-wide CpG island methylation analysis microarray

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CpG islands are stretches of high GC content DNA containing multiple CpG dinucleotides. When CpG dinucleotides within these islands are methylated, especially in promoter regions, expression of the corresponding downstream genes is often repressed. Aberrant CpG island methylation is implicated in cancer. We have refined a protocol for methylated DNA immunoprecipitation (mDIP) and coupled it with microarray detection. DNA isolated by mDIP is fluorescently labeled and hybridized to an oligonucleotide microarray that specifically represents the unique CpG islands in the human genome. This microarray contains ~237,000 oligo probes tiling ~20,000 CpG islands, with an average spacing between probes of 95 base pairs. In addition, we have tiled CG rich promoter regions. As proof of concept we perform mDIP and microarray analysis of human genomic DNA samples from normal tissues. We compare our mDIP array data with bisulfite sequencing data. We demonstrate the ability of our array based methylation assay to distinguish probes corresponding to methylated regions from probes corresponding to unmethylated regions. We then apply the whole-genome assay to tumor and normal DNA and identify differential methylation patterns.

**P11.028****Linking macro ncRNAs to human imprinted gene clusters and CIMP (CpG Island Methylator Phenotype) regions in normal and cancer cells**

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Genomic imprinting results in parental-specific gene expression and offers one of the best examples of an epigenetic gene silencing mechanism in mammals. The analysis of imprinted gene expression in mouse models has identified two important but unexpected, epigenetic mechanisms. First, that DNA methylation acts to silence macro non-protein-coding RNAs. Second, that macro ncRNAs act to silence flanking genes *in cis*. To date, two examples of imprinted macro ncRNAs with a silencing function are known (*Air* and *Kcnq1ot1*, reviewed in Paufer & Barlow 2006). In order to identify and characterize new macro ncRNAs we selected imprinted regions from the human genome and CIMP (CpG Island Methylator Phenotype) regions to generate HIRTA (Human Imprinted Region Tiling Array) Chips. RNA samples from different tissues, ES cells and from normal or tumor cell lines are hybridized to these chips using the RETA (RNA Expression Tiling Array) technique. In order to analyze the results from the Chips we have developed the NORBERT (NOnc- coding RNA identification Based on Enrichment on RNA Tiling array) program. Our goals are to test: (1) if macro ncRNAs are a common feature of imprinted regions in the human genome, (2) if imprinted macro ncRNAs are deregulated in cancer, and (3) if macro ncRNAs play a role in regulating non-imprinted genes. We are particularly interested to test if macro ncRNAs play a role in the gain DNA methylation of tumor suppressor genes in tumors. Our preliminary data show that by this approach we can identify 5 well-known imprinted ncRNAs and that we are able to detect novel macro ncRNA candidates. Our immediate goal is to characterize the transcriptional features of novel candidate macro ncRNAs.

**P11.029****Assessing the levels of CREB1 after siRNA mediated knockdown in K562 cells**

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CREB1 is an important downstream protein for many signaling pathways. By designing efficient siRNAs against CREB1, it may be possible to assess the role of molecules involved in signaling pathways in different cell types. In this research the efficiency of CREB1 knockdown by 2 different siRNAs in K562 cells have been studied. siRNAs have been designed according to the criteria suggested by Reynolds *et al.* K562 cells were transfected by siRNA using Lipofectamine 2000. The efficiency of CREB1 knockdown has been assessed by quantitative relative real time PCR. Our results have shown that only one of the siRNAs has a high level of inhibitory effect on CREB1 gene expression. The expression of CREB1 by this siRNA was knocked-down 79.5% in K562 cells. Reasons other than the aforementioned criteria may be involved in effectiveness of siRNAs.

**P11.030****Transcriptional profiling of mouse embryos with cardiac and thymic defects induced by antagonist of retinoic acid and recovered by supplementation with folic acid**

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Congenital heart diseases (CHDs) account for 25% of all human congenital abnormalities and affect 1-2% of newborn children. Specific malformations of the outflow portions of the heart are termed conotruncal malformations (CTHM; OMIM 217095) and account for a fourth to a third of all nonsyndromic congenital heart defects.

By induction of a retinoic acid competitive antagonist (BMS-189453)

we developed a mouse model of CTHMs (81.3%), thymic abnormalities (98.4%) and neural tube defects (NTD, 20.3%). A nutritive therapy based on folic acid (FA) administered to mouse embryos previously treated with BMS-189453, resulted in a reduction of CTHM (64.8%), thymic abnormalities (27.8%) and NTD (3.7%).

We performed a global transcription analysis by microarray to identify genes or molecular pathways affected in both the experimental models.

A total of 447 genes were differentially expressed (FC=  $\pm 1.5$ ) in BMS-treated mouse; while a total of 239 genes were differentially expressed in BMS+FA-treated embryos. A comparative analysis of these gene expression patterns revealed 140 common genes; 70 of them includes genes that were down or up regulated in BMS-treated embryos, but returned to a "wild-type" level in BMS + FA-treated embryos. These genes were mainly involved in protein metabolism (14.8%), transport (10.2%), signal transduction (13%), cell cycle (7.4%) and transcription (6.5%).

QRT-PCR assay performed on a selected group of commonly regulated genes confirmed the microarray data.

The discrete number of genes which resulted from our data might be considered as candidate genes for conotruncal heart and thymic malformations in humans.

**P11.031****The CYLD tumor suppressor sensitizes cells to microtubule destabilization**

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Mutations in the CYLD tumor suppressor have been identified in patients with familial cylindromatosis and familial trichoepithelioma, which are both autosomal dominant genetic predispositions to multiple tumors of the skin appendages. CYLD has been shown to deubiquitinate TRAF proteins and Bcl-3, both leading to inhibition of NF- $\kappa$ B activation. We have now found that CYLD is a microtubule-associated protein that accelerates microtubule destruction in cells treated with the microtubule-depolymerizing agent nocodazole. CYLD protein carrying a point mutation that truncates the protein at a.a. 485 and thereby deletes the C-terminus, including the majority of the third predicted CAP-GLY domain, still associates to microtubules, but has no influence on microtubule stability. Accordingly, specific knockdown of CYLD results in an increase of microtubule stability and faster recovery after nocodazole withdrawal. Our data strongly suggest that, in addition to upregulation of NF- $\kappa$ B signalling, microtubule dynamics plays an important role in the development of skin cancer induced by mutations in the tumor suppressor CYLD.

**P11.032****The integration of approaches for the study of biological networks in the inner ear**

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Systems biology involves studying the interaction and interplay of many levels of biological regulation. We combined comparative transcriptomic and proteomic analyses of early post-natal cochlear and vestibular sensory epithelia to identify networks of genes and proteins essential for the development and function of these inner ear organs. Expression profiling of vestibular and cochlear sensory epithelia was performed using Affymetrix microarrays. Proteomics analysis was performed using the Q-TOF mass spectrometer with iTRAQ labeling (Smoler Proteomics Center, Technion). Integration of the transcriptome and proteome data led to the identification of genes/proteins that may play an important role in the inner ear. These genes/proteins are being examined in further detail.

In addition, we identified microRNAs (miRNAs) that are expressed in these sensory epithelia using the miRCURY LNA array system. We integrated the transcriptome, proteome and miRNA levels to efficiently predict targets of miRNAs in the inner ear using newly developed algo-

rithms. Using this integration, the number of potential targets predicted using bioinformatic tools was significantly reduced. These targets are now being validated to determine whether they are true biological targets.

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#### P11.033

#### Ribosomal protein S19 and S24 insufficiency in Diamond-Blackfan anemia cause prolonged cell cycles with distinct arrests in non-hematopoietic cells

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Diamond-Blackfan anemia (DBA) is a severe congenital anemia characterized by a specific decrease of erythroid precursor cells. Although anemia is the most prominent feature, the disease is also associated with growth retardation and congenital malformations in 50% of patients. Heterozygous mutations in one of the seven ribosomal protein (RP) genes *RPS7*, *RPS17*, *RPS19*, *RPS24*, *RPL5*, *RPL11* and *RPL35a* have been identified in approximately 35% of patients. We established primary fibroblast cell lines from DBA patients with truncating mutations in the *RPS19* (c.72-2A>C) and *RPS24* (c.1A>G) genes. A growth assay showed that fibroblasts from DBA patients with truncating mutations have a marked reduction in proliferative capacity. Mutant fibroblasts are associated with cell cycles extended by 50% and 33%, respectively, when compared to w.t. cells. *RPS19* mutant fibroblasts accumulate in the G1 phase suggesting a G1/G0 arrest, whereas *RPS24* mutant cells are significantly reduced in the G2 phase. We also observe a concomitant down regulation of the small subunit proteins in mutant cells. The mutations result in impaired rRNA maturation and ribosomal subunit assembly. The results show that the major cause of impaired growth in *RPS19* and *RPS24* insufficient fibroblasts is a delayed cell cycle with distinct profiles. We suggest that the reduced proliferative capacity is an important contributing mechanism behind extra-hematological features in DBA.

#### P11.034

#### Comparison of DNA methylation patterns in three mouse tissue types.

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Enzymatic methylation of the cytosine residues in genomic DNA (gDNA) has been shown to correlate with gene expression. Methylation of cytosines (C) at CpG motifs, usually in the promoter regions of genes, will shut down expression of the gene in complex biological processes. Technologies like microarray and next generation sequencing allow identification of methylation patterns on a genome-wide scale but capillary electrophoresis analysis is ultimately used for detailed information of each CpG in the amplicon and remains the gold standard in validating DNA methylation results. In this study bisulfite treated gDNA of a specific gene region from three mouse tissues was cloned into pGEM-T vector, amplified and sequenced with BigDye® Terminator Cycle Sequencing v3.1 chemistry, and analyzed by capillary electrophoresis. A number of factors affect the reliability of methylation patterns revealed by sequencing including the purity of the gDNA, the efficiency of bisulfite conversion, and the interaction of modified DNA with primers and DNA polymerase. The extent of cytosine conversion by bisulfite is affected by the presence of protein associated with gDNA, the denaturation state of the target DNA and well as the quantity of DNA in the conversion reaction. In this study a control using methylated and unmethylated reference DNA is described that validates the bisulfite conversion conditions for improved reliability of the resultant methylation profile.

#### P11.035

#### Sequence Capture Approaches Coupled to Next Generation Sequencing to Identify Candidate Mutations Causing Inherited Disorders

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We have developed an optimized oligonucleotide microarray for genomic selection and targeted sequencing of the entire human CCDS database. The NimbleGen Sequence Capture 2.1M Human Exome array contains 2.1 million capture oligonucleotides targeting ~180,000 exons, totaling ~34 Mb of sequence. We have used this array, coupled to 454 Titanium sequencing technology on the Genome Sequencer FLX instrument, to sequence the exons of patients with idiopathic ataxia to ~10X average coverage. Data analysis has revealed previously uncharacterized mutations in genes known to cause neurological disorders. This approach has the potential to replace time-consuming and laborious classical genetic methods, and may lead directly to functional candidate mutations for highly penetrant inherited phenotypes.

#### P11.036

#### Comparative analysis of expression levels of genes of the extracellular matrix proteins from human normal and scar skin fibroblasts

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A major reason for scar formation is an abnormality in the synthetic activity of fibroblasts, which are the major producers of the extra cellular matrix (ECM) in different types of tissues. Compositional ratios of structural ECM proteins are strongly tissue-specific and variations in these protein compounds may contribute to architectural and functional heterogeneity. A particular kind of ECM organization is the epidermal basal membrane (BM). The main structural proteins of BM are laminin, collagen and fibronectin. In order to reveal the possible reasons responsible for scar skin formation, we compared the expression of genes encoding main proteins of the BM between normal, embryonic and scar skin human fibroblasts in culture. Using semi-quantitative RT-PCR method, we have estimated the expression levels of the following genes: LAMA2, LAMB1, LAMB2, LAMY1 encoding subunits of laminin; COLIA1 and COLIA2 encoding subunits of collagen type 1; and COLIVA1, COLIVA2, COLIVA4 and COLIVA5 encoding subunits of collagen type 4. The results showed that expression levels of genes LAMA2, LAMY1, COLIVA4 and COLIVA5 depended on type of skin fibroblasts origin. The expression levels of COLIVA4, LAMA2 and LAMY1 genes were higher in normal skin fibroblasts and the expression levels of COLIVA5 were lower in scar skin fibroblasts comparing to two other types of fibroblasts. No other correlations were revealed. We have also developed a sparing method of ECM proteins isolation from cultured fibroblasts. Our next goal is to estimate whether differences identified in genes expression on RNA levels also reflect different amounts of the corresponding protein products.

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#### P11.037

#### A Molecular Combing approach for FSHD: direct visualization of the FSHD locus in individual DNA molecules for straightforward diagnosis and genetic and epigenetic explorations

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Facioscapulohumeral dystrophy (FSHD) is the third most common muscular dystrophy, with autosomal dominant transmission. FSHD is associated to the contraction of a repeat array at the subtelomere of chromosome 4q (4q35), which comprises 1-150 copies of a 3.3 kbp repeat unit, D4Z4. A virtually identical array is present at the subtelomere of chromosome 10q. FSHD alleles carry 1-10 copies on a 4qA chromosome, one of two equally frequent variants of the 4qter subtelomere.

4qA arrays with more than 10 repeat units, as well as 4qB and 10q arrays, are non-pathogenic.

The current genetic diagnostics relies on Southern blotting, which allows sizing of D4Z4 repeat arrays and, to some extent, determination of their chromosomal origin and haplotype. However, it is cumbersome and cannot reliably account for relatively frequent deviations from the canonical description above, such as recombinations, translocations between 4q and 10q, deletions of sequences adjacent to the repeat arrays, somatic mosaicism etc. To bypass these heavy limitations, we have developed a test based on a novel approach, the direct visualization of the D4Z4 loci at kilobase-resolution on individual DNA molecules stretched by Molecular Combing.

Our results on reference cell lines and patient samples demonstrate the ability of Molecular Combing to precisely visualize the haplotype and provide the repeat unit counts of all four 4q- and 10q- D4Z4 arrays in a single experiment. Furthermore, we identified unexpected rearrangements at the D4Z4 loci. This approach should allow for routine genetic testing, as well as provide better understanding of the pathophysiology of FSHD.

#### P11.038

#### A unique tool to detect LDLR, APOB and PCSK9 point mutations as well as Copy Number variation of the LDLR gene

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Familial Hypercholesterolemia (FH) is an autosomal dominant disorder with a prevalence of heterozygous FH of one in 500 individuals. It is mostly due to point mutations in the low-density lipoprotein (LDL)-receptor gene (LDLR) and Copy Number Change (CNC) of part of this gene account for 5-10%. Mutations are also seen in the APOB and PCSK9 genes.

To improve FH genetic diagnosis, we have developed a new diagnostic tool based on a DNA-array system: Lipochip which is able to detect at the same time point mutations in the LDLR, APOB and PCSK9 genes as well as CNC in the LDLR gene.

To enable detection of CNCs, controls probes from the chromosomes X and 21 were included on the chip. 247 mutations were included on the LIPOchip (238 LDLR, 3 APOB and 6 PCSK9 mutations). A software was created for the automated analysis.

All point mutations and all types of CNC hybridized were identified. Only one discrepancy was observed between MLPA and the LIPOchip, due to a polymorphism in the sequence hybridizing the MLPA probe. Reviewing the prevalence of the different mutations in Europe, we estimate that this tool is able to detect more than 80% of the mutations of representative European populations (Spain, Norway, Netherlands). We here show that a unique tool is able to detect at the same time point mutations and CNCs allowing rapid and robust genetic diagnosis of FH.

#### P11.039

#### Analysis of the expression profile of mRNAs and microRNAs in fragile X syndrome.

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Fragile X syndrome (FXS) is caused by the absence of the FMRP protein. FMRP is involved in multiple pathways of mRNA metabolism and stability [Zalfa et al., 2007]. Indeed, FMRP has been shown to be associated to microRNAs (miRNAs) in mammals [Jin et al., 2004], possibly regulating neuronal mRNA translation or stability.

We performed microarray experiments to characterize the transcriptional profile of lymphoblastoid cell lines from 4 FXS, 3 controls and 2 unmethylated full mutation (UFM) carriers. Bioinformatic analysis yielded a list of genes either up- or down-regulated in FXS lymphoblasts, compared to controls. We selected 8 genes for validation and confirmed that 5 of these were actually differentially expressed. We also analyzed the miRNA expression profile in lymphoblasts from 6 FXS, 6 controls and 3 UFM carriers. We observed internal homogeneity in each of the three groups (FXS, controls and UFM) and confirmed

the inter group difference ( $p < 0.05$ ). We identified few differentially expressed miRNAs in the three groups and found that some corresponding mRNA targets were accordingly modified, as shown by the mRNA profiling experiments. Interestingly, 5 of the selected miRNAs also bind to the 3' UTR of the *FMR1* gene.

These experiments may lead to the identification of FXS pathogenetic pathways, eventually allowing pharmacological therapy of FXS, in spite of the absence of FMRP.

#### P11.040

#### MBD2 mediated $\gamma$ -globin repression in K562 cells is not dependent on its association with LARC

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The methyl binding domain protein (MBD2) acts as a repressor on the human  $\gamma$ -globin gene. In transgenic mice harboring a human  $\beta$ -locus, knockout of MBD2 leads to a substantial induction of  $\gamma$ -globin mRNA (Rupon et al. *PNAS*, 2006). However MBD2 does not seem to act by binding to CpG regions in the  $\gamma$ -globin promoter. Here we have sought to determine whether MBD2 is mediating its effect through association with the LCR-associated remodeling complex (LARC)\_a complex that contains NURD (and MBD2) and binds to the LCR as well as the  $\gamma$ -globin promoter (Mahajan et al. *PNAS*, 2005). Here we have first determined that K562 cells are an appropriate model system to study MBD2-mediated  $\gamma$ -globin repression. In particular, we have shown that a validated siRNA targeting MBD2 leads to a 35% knockdown of MBD2 and a corresponding 1.7-fold increase in  $\gamma$ -globin in K562 cells (using qPCR and GAPDH as the internal control). In contrast, a validated siRNA targeting hnRNP C (C1/C2), the DNA binding protein of LARC, gave a 46% knockdown of hnRNP C but had no appreciable effect on  $\gamma$ -globin RNA levels (1.07 relative to a scrambled siRNA). These results suggest that MBD2 mediated  $\gamma$ -globin repression is not mediated through LARC in K562 cells. We are in the process of using lentiviral vectors to deliver MBD2 and hnRNP C shRNAs. Delivery via transduction followed by antibiotic selection should lead to better knockdown levels. This should help us more accurately determine the role of these proteins (if any) on  $\gamma$ -globin transcription.

#### P11.041

#### Detecting known and novel splicing events in the human transcriptome by high throughput sequencing

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Alternative splicing of exons is a major factor in promoting diversity of cell type and function from a limited set of genes. Recent studies have shown that alternative splicing is far more common than previously thought with almost all human genes transcribing multiple isoforms. Traditionally, Sanger sequencing of full cDNA clones as well as exon microarrays have been used to identify alternative splicing events. More recently next-generation sequencing is emerging as a cost effective method to infer splicing by mapping tens of millions of sequencing reads to the human genome.

Current approaches to map sequencing reads are limited to finding known and novel alternative splicing events between annotated exons. Here, we present a method capable of finding known and novel alternative splicing events between known and novel exons. The method is capable of identifying inter-chromosomal fusion transcripts as well.

We applied this method to transcriptome sequencing data generated on the Applied Biosystems SOLiD™ System from a library prepared from poly-A purified MAQC Human brain reference (HBR) RNA and Human universal reference RNA. For HBR, of the 17,320 genes transcribed, 67% are found to have alternative splicing events between known exons. Only 39% of these transcripts are described in the RefSeq database. Further, we found 93% of expressed genes participating in alternative splicing when events between known exons and unannotated exons are included. A further 1,544 events between unannotated exons are also found. We are conducting additional experiments to validate these findings.

**P11.042****A digital anatomical atlas of the murine transcriptome at embryonic stage E14.5****G. Diez Roux, EURExpress consortium;***Telethon Institute of Genetics and Medicine, Napoli, Italy.*

We have generated the first genome-wide digital transcriptome atlas by RNA *in situ* hybridization (ISH) of the developing mouse at embryonic stage E14.5. The expression patterns of approximately 18,000 genes were organized, curated manually and placed in a specifically designed database freely available to the scientific community ([www.eurexpress.org](http://www.eurexpress.org)). This database allows browsing, downloading and searching the data by keyword, gene sequence and by anatomical site of expression. To generate the digital atlas we produced 20,000 templates and generated 360000 ISH images. The manual annotation of the data describes site, strength and pattern of expression using a specifically designed ontology that includes over 1400 anatomical structures. The global analysis of gene expression annotation determined that 39% of genes displayed a regional or restricted expression pattern at this stage. We found that about 950 genes, 16% of which are of unknown function, display exclusive expression to particular anatomical structures. We used the textual annotation to perform data clustering analysis and we found several examples of synexpression gene clusters. The quality and the resolution of the data allowed performing high-resolution molecular regionalization, uncovering a series of new markers for several developing structures. In conclusion, EURExpress is the first genome-wide digital transcriptome atlas that gives a panoramic view of gene expression in an entire mammalian organism and represents a unique resource to uncover many novel ontogenetic and functional associations relevant to development and disease.

**P11.043****Evaluation of Common Gene Expression Patterns in Colorectal Cancer****M. Ioana<sup>1</sup>, C. Soare<sup>1</sup>, F. Burada<sup>1</sup>, C. Gug<sup>2</sup>, E. Cioboa<sup>1</sup>, F. Mixich<sup>1</sup>, M. Cruce<sup>1</sup>;**<sup>1</sup>*Univ Med Pharm Craiova, Craiova, Romania, <sup>2</sup>Univ Med Pharm Victor Babes Timisoara, Timisoara, Romania.*

**BACKGROUND:** DNA damage checkpoint is one of the surveillance systems to maintain genomic integrity. The aim of our study was to compare genes expression profiles in normal, premalignant and malignant specimens in order to distinguish differentially expressed genes by using quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR).

**PATIENTS AND METHODS:** In order to detect the gene expression patterns we analyzed three sample types for each of our 25 patients: specimens from diverse sites of healthy gut, adenomatous polyps and malignant tissue. In order to asses the RNA quality we analysed the 18S and 28S ribosomal RNA bands integrity by electrophoresis. The reverse-transcription was performed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). In order to evaluate gene expression, we used Applied Biosystems validated TaqMan Gene Expression Assays for 9 genes associated with the ATR/ATM signaling network and transcriptional targets of DNA damage response.

**RESULTS:** We successfully performed qRT-PCR analysis showing that a dysfunction in DNA damage response contributes to genomic instability in colon samples. In 20 of our malignant samples we detected a significantly disregulation of gene expression in six DNA repair genes (ANKRD17, EXO1, MLH1, MLH3, MSH2, MSH3) comparing with the normal specimens.

**CONCLUSION:** Determination of gene expression profiles by using qRT-PCR is an ideal tool to improve knowledge of CRC molecular pathways. However, defined gene signatures are highly variable among studies, none of the identified expressional patterns or molecular markers has been successfully validated as a diagnostic or prognostic tool applicable to routine clinical practice.

**P11.044****The multiple alternative transcripts of human sphingomyelin synthase 1 (SMS1) gene****A. V. Rozhkova, V. G. Dmitrieva, A. S. Konkov, S. A. Limborska, L. V. Dergunova;***Institute of Molecular Genetics RAS, Moscow, Russian Federation.*

Recently sphingomyelin synthase 1 (SMS1) gene has been identified. The enzyme SMS1 occupies a central position in sphingolipid metabo-

lism: it catalyses the conversion of ceramide and phosphatidylcholine to sphingomyelin and diacylglycerol (DAG). Since SMS1 may have a direct impact on cell proliferation and life span by regulating the cellular levels of pro-apoptotic factor ceramide and myogenic factor DAG it is of a great importance to investigate the function of SMS1 gene in the cell. To date we have found 12 new alternative transcripts of SMS1 gene using *in silico* and *in vitro* methods. Most of them differ from SMS1 mRNA in 5'-untranslated regions (5'-UTR). New-revealed 5'-UTRs are located in front of exon I and also between exons I and II, II and III, VI and VII. These exons were identified in SMS1 gene earlier. We also found alternative transcripts of this gene that include not-known before exons, situated between exons VII and VIII. Analysis with GENOMATIX software revealed several putative promoter regions adjacent to four new exons. Variations in levels of different transcripts between tissues were obtained by semi-quantitative RT-PCR. We presume that regulation of SMS1 gene functional activity is provided by using alternative promoters and synthesis of alternative mRNAs. It was shown that some of predicted open reading frames of new transcripts encode full-length SMS1 protein while others encode C- or N-terminal truncated forms of the protein. In particular circumstances the presence of different alternative transcripts of SMS1 gene may give rise to at least three different proteins.

**P11.045****Establishing a genotyping platform for pharmacogenetic investigations in anesthesia: GeneChip resequencing array****S. Levano, M. Singer, A. Urwyler, T. Girard;***University Hospital Basel, Basel, Switzerland.*

In anesthesia two inherited pharmacogenetic diseases are of primary interest: malignant hyperthermia (MH) due to the potentially fatal consequences and butyrylcholinesterase (BCHE) deficiency due to its high incidence. MH is a dominantly inherited disorder of skeletal muscle triggered by inhalative anesthetics in genetically predisposed individuals. MH susceptible (MHS) patients are known to carry mutations in RYR1 and CACNA1S genes. Because of the large gene sizes, minor allele frequency of causative mutations of RYR1 gene as well as lack of predominantly hot spot regions in the CACNA1S gene, the acquisition of genetic information in a routine way is difficult. Mutations in the BCHE gene can substantially prolong the duration of action of short acting neuromuscular blocking drugs. This prolongation prevents the patient from spontaneous ventilation for hours. We aim to maximize the information of these anesthesia related genes in a practicable way applying array technology. The development of the genechip included the selection of the gene sequences, selection of the positive SNP controls, setup of the PCR parameters, verification of the PCR fragments, labeling of purified PCR fragments and finally chip hybridization and analysis. We will present the preliminary data obtained using this chip. The resequencing based array technology enables sequencing a large number of bases on a single chip. This resequencing array promises a reliable, efficient and high throughput method that would improve the analysis of these heterogeneous genes and therefore facilitate to determinate the genetic basis of MH susceptibility and contributes to safer administration of drugs.

**P11.046****Genetic diversity of the Russian populations of genes of rare hereditary disorders****R. A. Zinchenko, G. I. El'chinova, N. V. Petrova, L. A. Bessonova;***Research Center for Medical Genetics, Moscow, Russian Federation.*

The genetic diversity of genes of monogenic hereditary disorders (HDs) in 11 populations of Russia (6 ethnic groups: Russians 6 regions, Maris, Chuvashs, Udmurts, Adighes, Bashkirs) was analyzed. The size of the investigated population was 2.8 millions of persons. All population was examined by standard protocol of medical genetic research elaborated in laboratory of genetic epidemiology, Research Centre for Medical Genetics. About 2500 HDs of OMIM could be identified by this research. Clinical investigations were performed by neurologists, ophthalmologists, orthopedic, otolaryngology's, dermatologists, pediatricians and clinical geneticists, focused on diagnostic of HDs. Simultaneously with medical genetic study the population genetic study was performed in the populations. By comparing both studies it was suggested that the genetic drift is a most important factor which determines genetic differentiation of populations by the prevalence and ge-

netic diversity of autosomal disorders. Genetic diversity of HDs in the investigated populations was revealed 454 disorders (6800 affected): 215 AD, 183 AR and 56 X-linked recessive. It is established, that 57.39 % of patients for all types of inheritance concern to the common forms of the HDs causing only 6 % of all revealed nozological forms. These 28 disorders meet with high frequencies in all investigated populations (15 with AD, 8 with AR and 5 with X-linked inheritance). However, besides these 28 diseases which frequencies also varied between regions, specific common diseases are revealed in each population. It has been demonstrated that the genes of HDs are a promising tool for characteristic ethnogenetic processes in populations.

#### P11.047

#### HGVbaseG2P: an advanced database for the integration of genetic association datasets

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The reporting of genetic association studies is far from optimal since in many cases (especially negative findings) results are not made publicly available. Consequently, it is very difficult to determine what signals should be believed, or to integrate the results of different studies. To improve on this situation, the Human Genome Variation Genotype-Phenotype database (HGVbaseG2P: [www.hgvbaseg2p.org](http://www.hgvbaseg2p.org)) has been constructed to provide a new publication medium for association study results - including large-scale, small-scale, GWAS, candidate gene, positive, and negative findings. HGVbaseG2P focuses upon summary-level information, and work is underway to orchestrate the control of access to such datasets globally, to overcome concerns about identifying study participants from such information.

HGVbaseG2P aims to combine the best features of a database and a scientific journal, and in that respect a partnership has been established with HUGO and Springer to explore awarding DOIs and PubMed IDs to studies submitted to HGVbaseG2P. Additionally, curators actively gather and collate data from all relevant public data resources.

The latest version of HGVbaseG2P provides a highly-innovative set of new genome browser options for visually comparing and contrasting genetic association datasets - both at genome wide and region specific levels.

To disseminate these technologies more widely, a blank 'HGVbaseG2P-in-a-box' will shortly be made available so that others can create compliant databases to report their own compilations of research findings. All such databases will automatically be interoperable, enabling pan-database searches.

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#### P11.048

#### GEN2PHEN Knowledge Centre: a virtual centre of excellence for the genotype-to-phenotype community

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The GEN2PHEN project aims to help establish increasingly holistic access to Genotype- Phenotype (G2P) information. This goal involves promoting a federated network of G2P resources, and enabling the bi-directional flow of knowledge between public G2P databases to G2P researchers. To this end, we are constructing the 'GEN2PHEN Knowledge Centre' (GEN2PHEN-KC: [www.gen2phen.org](http://www.gen2phen.org)).

The GEN2PHEN-KC will be much more than a simple informational website, as it will provide: i) increasingly comprehensive searches of broad segments of the G2P domain, ii) systems by which researchers can add comments onto records in public databases, allied to various modes for the community to discuss small or large topics in the G2P field, iii) access to software, tools and resources created by the GEN2PHEN Project, iv) latest news and updates on the G2P field, including an extensive diary of relevant events, v) training resources, such as tutorials, screencasts and instructional videos.

Such features will equip researchers with an easy-to-use and effective central outlet to promote their research, to network and establish collaborations, and to assess the value of G2P datasets and resources. Researchers will also be able to rate and comment on articles, blog posts and resources, edit wiki-type pages, and engage in debates with peers.

The first version of the website provides an introduction to the GEN2PHEN project, and this will evolve into the fully functional GEN2PHEN-KC during the first few months of 2009, with ongoing development thereafter.

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#### P11.049

#### UGT1A1(TA)n promoter polymorphism in Spanish patients with a clinical diagnosis of Gilbert Syndrome

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Gilbert's syndrome is a mild hereditary unconjugated hyperbilirubinemia caused by mutations in the UDP-glucuronosyltransferase gene (UGT1A1). The causative mutation in Caucasians is almost exclusively a TA dinucleotide insertion in the TATA box of the UGT1A1 promoter. The vast majority of affected individuals are homozygous for the variant promoter and have 7 instead of 6 TA repeats.

The aim of the present study was to determine the genotypes of UGT1A1(TA)n promoter in 686 cases referred to our laboratory with clinical diagnosis of Gilbert Syndrome.

To determine the number of TA repeats, we performed a fluorescence-labelled PCR. The PCR products were separated by an automated capillary electrophoresis and analyzed using the ABI GeneScan programme (Applied Biosystems).

We have identified the following genotypes: TA<sub>5</sub>/TA<sub>6</sub> (n:1); TA<sub>6</sub>/TA<sub>6</sub> (n:58); TA<sub>6</sub>/TA<sub>7</sub> (n:147); TA<sub>6</sub>/TA<sub>8</sub> (n: 5); TA<sub>7</sub>/TA<sub>7</sub> (n: 472); TA<sub>7</sub>/TA<sub>8</sub> (n: 2) and TA<sub>8</sub>/TA<sub>8</sub> (n:1).

The TA<sub>8</sub> allele was first described by Beutler in subjects with African ancestry and is very rare in Caucasians. Four cases out of five with a TA<sub>6</sub>/TA<sub>8</sub> genotype were first degree relatives of the boy with a homozygous TA<sub>8</sub>/TA<sub>8</sub> genotype. This is, to our knowledge, the first homozygous case of this rare allele.

#### P11.050

#### Complete sequencing of the CFTR gene using next-generation GS-FLX sequencing technology

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Next generation sequencing technologies have been recently introduced. However, this technology was initially developed for whole genome sequencing purposes.

We have adopted this technology for complete sequence analysis of the CFTR coding region.

For a 50x coverage, only half a million nucleotides are needed for CFTR sequence analysis of one patient. Therefore, 100-200 samples should be pooled in order to use the full capacity of the GS-FLX system. We have developed an economically feasible universal sample tagging approach allowing the pooling of 100 samples with one set of 260 primers (60 amplicon-specific primers and 200 tagging primers). This compares to 6000 primers if amplicon-specific primers are tagged as such.

Normally, each amplicon should be amplified individually and purified. Then, the concentration of each solution is accurately determined in order to pool all amplicons in equimolar concentrations. The universal tagging approach resulted in a less dynamic range of the yield of the different amplicons, so that the time-consuming preparation of equimolar solutions could be simplified.

We are even further simplifying the PCR steps through the development of robust multiplex PCR reactions. Indeed, 30 amplicons should be analyzed for the CFTR gene, and this can ultimately be only economically feasible if amplified in one, or a limited number, of multiplex PCR reaction(s). Specifically, we have developed a robust multiplex amplification assay in which biotinylated amplicon-specific primers are locally restricted through streptavidin/biotin crosslinking.

We thus developed an assay for routine sequencing of the CFTR gene in a diagnostic setting using next generation sequencing.

**P11.051****Allelic spectra of large genomic regions identified by DNA pooling, array capture and high throughput sequencing**

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Genome wide association studies (GWAS) typically report regions with common tag variants, variably correlated with the actual biologically causal genetic variants. Additional, independently associated, rare and common variants may exist. To identify the spectrum of genetic variants at three celiac disease associated genomic regions (total 1.26Mb, of which 1.01Mb non-repeat sequence was tiled and analysed), we made 10 pools of 8 human DNA samples and enriched each pool using Nimblegen sequence capture microarrays and high-throughput sequenced captured fragment paired-ends. We developed variant calling algorithms and identified 3457 SNPs (45.8% previously known), 517 small ( $\leq 7$ bp) insertion-deletions variants (25% previously known) and over 10 larger structural variants by read-pair insert analysis. A 4.98% per-SNP false negative rate was determined using sample Hap300 BeadChip genotypes. A 6.1% per-SNP false positive rate was determined using deep dideoxy capillary resequencing of 52 randomly selected SNPs. These methods will advance identification of causal variants from GWAS regions.

**P11.052****Influences of genetic variants on plasma factor VIII levels in female carriers of hemophilia A**

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Introduction: An inversion in intron 22 of the factor 8 gene causes severe hemophilia A with FVIII:C activity (FVIII:C)  $<1\%$ . In female carriers FVIII:C is highly variable. Age, blood group and von Willebrand factor antigen (vWF:Ag) concentration could influence FVIII:C. Currently, data on their effect on FVIII:C in female carriers are not available. Also, systematic analyses of the role of the inversion in intron 22 and X-chromosome inactivation (XCI) on FVIII:C are lacking.

Patients and Methods: We measured FVIII:C by one stage assay in 48 female carriers - 21 with inversion 22. Blood group and vWF:Ag were determined using standard methods. XCI was analyzed by PCR amplification of the human androgen receptor (HUMARA).

Results: FVIII:C ranged from 14 to 192%, the vWF antigen varied from 63 to 236%. We found no influence of age. Carriers with blood group 0 showed lower FVIII:C (mean 76%) than carriers with non 0, the difference was not significant. XCI could be evaluated in 36/48 individuals. Fourteen carriers had a balanced XCI pattern while 22 showed skewing. In 12 the ratios of  $X_1:X_2$  were 1 to 3 or higher. Skewing was equally frequent in inversion carriers ( $n=7$ , 32%) and non-inversion carriers ( $n=7$ , 27%). FVIII:C was not affected by skewing. The inversion in intron 22 did not influence FVIII:C ( $p=0.969$ ).

Conclusion: Age and blood group had no significant effect on FVIII:C in female carriers. Even though skewed XCI is frequent, an association of FVIII:C with skewing was not found.

**P11.053****Detection of genetic determinants relevant for HCV pathogenesis**

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Hepatitis C Virus (HCV) is a successful pathogen that establishes a persistent infection in more than 70% of those who contract it. Chronicity is often associated with liver cirrhosis and carcinoma. Current treatment is effective in approximately 50% of patients, likely due to host genetic factors.

Human genetic variability ranges from point-form variations (SNPs) to large genetic rearrangements, such as Copy Number Variants (CNVs). Since CNVs represent the amount of copies of a particular DNA fragment, they are currently emerging as a potential source of susceptibility to a significant panel of both multi-factorial and infectious disorders. We are focusing on the chromosomal determinants related with the pathogenetic consequences of chronic HCV infection, hunting for genetic aberrations relevant for both the progression of hepatic damages and the therapeutic outcome. The recruitment of blood and liver biopsies from HCV-infected patients is performed according with the liver fibrosis level and the therapeutic response. Hundreds of genomic DNA samples have been already subjected to Whole Genome Association (WGA) studies employing the microarray technology developed by Illumina®. In order to assess SNPs and CNVs associated to the HCV infection, microarray data are analyzed with informatics and statistical devices, such as Nexus, QuantiSNP and R. Validation of any genetic association disclosed by the WGA is being confirmed by an independent sensitive analysis based on both High-Resolution Melt and Real-Time PCR. Our study can help to improve the actual knowledge on the host susceptibility to HCV, shedding a new prospective on the infection outcome and therapeutic possibilities.

**P11.054****GLEAM: a possible solution to the problem of analysing heterogeneous disorders**

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Analysis of heterogeneous disorders is laborious and expensive. GLEAM is a tool for researchers and diagnostic laboratories wishing to eliminate known causative genes. It can be used for autosomal dominant, recessive and X-linked conditions. The use of GLEAM enables researchers to identify those families in which unidentified genes are likely to be causative, allowing further study of these cases. In a diagnostic setting it will reduce the number of causative genes requiring sequencing in families in which mutations are sought and potentially identify large scale deletions. Mutation specific oligos can also be incorporated within the analysis.

The proof of principle used CHIP based technology and required DNA from only two affected relatives from families in which the causative gene was known. It eliminates the need for samples from multiple family members and a good family structure which makes conventional linkage analysis difficult in most cases. Crucially there is no need to establish phase.

The level of informativeness is determined by the degree of relationship between the individuals used and was observed to be 60% in the pairs chosen for the pilot study. This agreed with our predictions based on the relationships. Where they were informative the correct gene was eliminated in all cases. This demonstrates the potential for reducing the need for sequencing and the laborious analysis of variants of unknown significance. Results from the use of GLEAM in breast cancer and cardiomyopathy will be presented.

**P11.055****Systematic comparison of High-Res Melting® genotyping methods on the LightScanner and LightScanner 32: Small Amplicons, Lunaprobes™, and Snapback Primers**

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Small Amplicon Genotyping (SAG) and LunaProbes are well established High Resolution Melting genotyping methods. SAG requires two primers whereas LunaProbes require an additional 3' blocked oligonucleotide. A new method, Snapback primers, shows promise as a genotyping technique. Snapback uses two primers, with one primer modified 5' with a "probe" element complimentary to the SNP region. Lunaprobes and Snapback produce a probe-melt (low Tm) and an amplicon melt (high Tm) region, while SAG produces a single amplicon

melt only.

Ten SNPs were interrogated using each method. Comparisons included genotype accuracy, discrimination of unequal allele fractions, and simplicity of design. SAG assays used primers placed as close as practical to each SNP, generating 50-70 bp amplicons. LunaProbes and Snapback utilized the same primers, generated 90-150 bp amplicons, and were designed to work using asymmetric PCR. LunaProbes were designed 18-35 bp. Snapback used one primer that was modified with a 5' probe element.

Each method was 100% accurate in genotype calls across all targets, with all genotypes represented. SAG assays were easiest to design and optimize. LunaProbes and Snapback required the lowest software sensitivity setting to call accurate genotypes, and displayed greater discrimination of unequal alleles, down to 5% of the low-fraction allele. All three applications are accurate, cost-effective and offer different advantages. Together, these methods will offer increased flexibility and success rate of assay development. SAG is the simplest method, while LunaProbes and Snapback offer greater ability to distinguish unequal allele fractions.

#### P11.056

#### A PCR coupled high-resolution melting analysis for reliable gene scanning of the facio-genital dysplasia gene, FGD1

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It is important to establish an easy and reliable system to detect mutations or variations for genetic testing. High-resolution melting analysis (HRM analysis) is a method, which allows simple and rapid detection of gene variations. We constructed a sensitive system for detecting gene variations in FGD1. The FGD1 gene is a responsible gene for Aarskog-Scott syndrome (AAS), which is an X-linked disorder characterized by short stature, dysmorphic facial appearance, brachydactyly, shawl scrotum, and sometimes neurobehavioral impairment.

We set up a PCR coupled HRM system for all exons of FGD1 using LightCycler 480 Instrument (Roche). Then we evaluated the PCR/HRM in the screening seven mutations of FGD1, which we found in AAS patients previously, plus variations of FGD1 in five sporadic patients, two families, and 48 controls. The PCR/HRM discriminated all the FGD1 mutations studied from wild-type DNA. In control individuals, four polymorphisms and three unknown variations were found in the FGD1 gene. Besides, the PCR/HRM discriminated not only four haplotypes in exon 14, but also between heterozygous and hemizygous or homozygous of those haplotypes.

The system is a valuable method for rapid and reliable scanning of FGD1 gene variations and is applicable to high-throughput genetic testing.

#### P11.057

#### Functional study of nuclear missense mutations causing mitochondrial HMG-CoA synthase deficiency

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The HMGCS2 gene codifies the human mitochondrial HMG-CoA synthase (mHS). The mitochondrial HMG-CoA synthase deficiency (OMIM 600234) is a rare autosomic recessive disorder of the ketone-bodies synthesis that sometimes cause sudden infant death. Up to date, eight patients with this deficiency have been reported. Clinical diagnosis must be confirmed by measuring the enzyme activity in liver tissue. Recently, we have cloned the HMGCS2 gene into the pMAL-c2x expression vector, overexpressed the recombinant plasmid into the *E. coli* BL21 and purified the mHS protein with amilose affinity chromatography. Here, we reported for the first time the obtention of the mHS protein correctly folded and with a great level of purity which allowed us to carry out accurate enzyme activity measurements. We have also studied the natural mutants of the enzyme and observed significant structural abnormalities in four of them. We identified two missense mutations that caused the complete loss of the enzyme activity and one missense mutation that caused the loss of 75% of enzyme activity.

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#### P11.058

#### Genomic targets of human papillomavirus E2 protein

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Papillomaviruses are DNA tumour viruses that infect epithelial cells and induce the formation of benign hyperproliferative lesions. HPV E2 protein regulates viral gene transcription and is required for viral DNA replication. E2 is a sequence-specific DNA-binding protein that recognizes a palindromic sequence 5'-ACCGNNNNCGGT-3' (E2BS). Our aim was to study the involvement of papillomavirus E2 protein in regulation of cellular gene expression. We have identified the abundance and placement of E2BS in the human genome and studied the role of some of these sequences in E2-dependent cellular transcription regulation. Bioinformatics analysis revealed that human genome contains over three thousand copies of E2-specific DNA motifs, but only 753 of them are located in repeat free regions. E2BSs occur less frequently than is expected from genome nucleotide content. Additionally, most sites are suboptimal for HPV E2 binding suggesting that the number and structure of these sites appears to be under negative selection pressure. Our experiments show that some of the genomic sequences containing at least two E2BS within 500 bp can act as E2-responsive transcription regulatory elements in transient transcription assays. E2 can also induce changes in expression levels of genes containing those sites in the genomic context. These data suggest that HPV E2 proteins can alter cellular gene expression through binding to specific E2 recognition sites in the human genome.

#### P11.059

#### Primer optimization for detecting low levels of methylation using High-Resolution Melting analysis

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DNA methylation plays a critical role in the regulation of gene expression in development, differentiation, and disease. Methylation of CpG Islands in promoter regions usually turns off gene transcription. Global hypomethylation of genomic DNA has been observed in tumor cells and a correlation between hypomethylation and increased gene expression has been reported for many oncogenes.

Most of the techniques available to study DNA methylation do not offer enough sensitivity to confidently detect methylated DNA levels less than 10%. HRM is a powerful method, able to achieve detection levels below 10%; however, knowledge of different parameters that influence detection sensitivity will improve the chance of a successful assay.

In this report, methylation of the promoter region of the MTA1 gene is examined using HRM. Positive controls with 100% and 0% methylation are used to establish melt curves for complete hyper- and hypomethylation, respectively. Unknown samples with as low as 0.1% methylation are detected using the HRM method. This can be achieved by including CpG dinucleotides in the PCR primer sequence and deliberately shifting the PCR bias to preferentially amplify the methylated sequence. The methylation status of each CpG is confirmed by directly sequencing the PCR product of the HRM reaction. These results demonstrate the sensitivity of HRM analysis in detecting extremely low levels of methylation, and the general workflow of sequencing the PCR product following HRM methylation analysis.

#### P11.060

#### Rapid high throughput screening and identification of unknown DNA variation using High-Resolution Melt and Sequencing workflow.

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High Resolution Melt (HRM) is a recent enhancement to traditional melting analyses that significantly increases the detail and information that can be captured. HRM requires the use of new DNA binding dyes that allow a sharp melting transition of double stranded to single stranded DNA. The HRM screening method is sensitive enough to detect single nucleotide differences in several hundred base pairs of sequence. We have successfully used HRM to screen for unknown DNA variations in genomic DNA. Traditional methods of DNA variant screening (dHPLC, SSCP) are more costly and time consuming than the HRM method. HRM screening identifies samples containing

sequence variation relative to a control sample; however, HRM does not impart the identity of the variation, and sequencing is required to exactly identify the nucleotide changes. Since HRM is an end-point PCR analysis technique, we further developed a workflow enabling the product of HRM PCR to be used directly in a Sanger sequencing reaction after a simple 100 to 200 fold dilution. The direct sequencing approach conserves sample by eliminating another round of PCR to generate product for sequencing. And the high ratio dilution eliminates the need for a purification step after PCR. The fluorescent dye used for HRM does not interfere with the sequencing reaction. To further simplify the workflow, universal M13 tags were added to the 5' end of the gene-specific primers allowing the HRM amplicons to be easily sequenced using a universal protocol in any high throughput sequencing setting.

#### P11.061

#### Intra-intronic human minisatellite UPS29 associated with neurological diseases regulates reporter gene activity depending on its copy number in cell line F9

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Earlier we found the association of increased rate of short alleles of minisatellite UPS29 localized in intron of *CENTB5* gene with some forms of Parkinson disease and epilepsy. The molecular mechanism of such correlation remains mainly unclear. To elucidate this problem we generated PCR product corresponding to "healthy" (900bp) and to "disease" (400bp) UPS29 alleles. These PCR products were introduced into plasmid pEGFP with ROSA-betaggc26 promoter and fused to reporter gene *GFP*. These plasmids were transfected into cells of embryonal carcinoma line F9. Number of GFP-positive cells, their morphology and fluorescence intensity were analyzed with the help of confocal microscopy after cultivation for 24 and 48h. Plasmids without UPS29 served as controls. There were no signal-positive cells in case of the same but minisatellite-free plasmids, though molecular analysis shower the presence of plasmids in F9 cells. Thus an GFP expression lacked in control, but appeared due to presence of UPS29 in construct. The number of GFP expressing cells depended on repeated units number in UPS29. The most high fluorescence was observed in neuron-like derivatives of F9 cells induced to differentiate. We suppose that such regularity could be explained by minisatellite-specific TF binding, and as a result UPS29 served as enhancer relatively to ROSA26 promoter. Obtained results point to possible mechanism of regulatory function of intra-intronic and other non-protein coding repeats as additional enhancers. The shortening of one of minisatellite allele will lead to gene expression decrease and further to pathology. Acknowledgement. The work was supported by RFBR grant 08-04-12167

#### P11.062

#### Epigenetic study on regulation of normal and mutant kit gene in mice

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The Kit protein is a tyrosine-kinase receptor with a key-role in normal development of Interstitial Cajal Cells (ICC), the kit gene having different expression levels in various tissues and developmental stages. We report preliminary results of an epigenetic study on the kit gene regulation in order to find correlation (if any) between gene expression level and methylation status of its regulatory elements in mice, in different tissues and organs in which ICCs were identified.

Different segments of gastrointestinal tract and extra-digestive organs were sampled from mutant B6Cg-Kit<sup>W<sup>sh</sup></sup>/HnihrJaeBsmJ and control B6129PF2/J mice. RNA and DNA were extracted by DNA/RNA Mini Kit (Qiagen). Gene expression analysis was performed by Reverse Transcription-PCR methodology. The methylation status of the kit regulatory elements was studied by both enzymatic restrictions with methylation-sensitive BstUI endonuclease followed by PCR and Methylation Specific-PCR after bisulfitic treatment of DNA by EpiTect® Bisulfite Kit (Qiagen).

Our study revealed comparable levels of the kit gene expression in

both mouse strains, high expression level being detected in bone marrow, spleen, liver and the lowest level in lung. Particular methylation pattern in a regulatory region (between -990 bp and -822 bp up-stream to the coding region) of the kit gene was identified in spleen, which may be correlated with high gene expression level. Our results suggest that DNA methylation may play an important role in tissue and organ-specific regulation of the kit gene, therefore our epigenetic study will be extended to other kit gene regulatory elements.

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#### P11.063

#### Colocalisation of predicted exonic splicing enhancers in *LAMA2* gene with reported sequence variants

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Translationally silent mutations were classified as polymorphisms. This assumption is now being challenged through the analysis of the mRNAs produced from mutant alleles, leading to the realization that a higher proportion of polymorphisms affect splicing. In our study, we analysed the colocalisation of exonic SNPs in *LAMA2* gene related to the MDC1A form of congenital muscular dystrophy with exonic splicing enhancers (ESEs). Then, we searched the effect of allelic change on ESEs efficacy. The *LAMA2* sequence was searched for ESE motifs using the web-based tool ESEfinder. Exons were screened for sequence motifs likely to be recognised by the SR proteins SF2/ASF, SC35, SRp40 and SRp55. Matching sequences are scored and only scores above thresholds are predicted to act as ESEs. Results showed the presence of 2709 ESEs in the 65 coding exons of *LAMA2* gene; this number was reduced to 480 significant ESEs after applying the thresholds values filter. Secondly, the analysis of published sequence variations in *LAMA2* gene showed the presence of 41 exonic SNPs, 18 of them colocalize with the identified significant ESEs, representing a fraction of about 44%. In addition, the allelic changes in 5 synonymous or non synonymous SNPs can abolish or create an ESE suggesting their potentially functional role in *LAMA2* gene expression. These results indicate the utility to consider the functional role of exonic SNPs in splicing and can also answer to many questions about the disease susceptibility and about the phenotypic variability observed in patients sharing with the same mutation in *LAMA2* gene.

#### P11.064

#### The Utility of Capillary Electrophoresis and Next Generation Sequencing platforms for scientific discovery

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With its long read lengths and high accuracy, capillary electrophoresis-based sequencing is the gold standard technology for *de novo* projects. Typically in *de novo* projects for genetic analysis of any organism CE is considered ideal for creating high quality scaffolds. Now with the availability of sequencing by short-read next generation sequencing technologies system, this process is complemented through finishing with high coverage. Alternatively, short-read sequencing technologies offer the throughput requirement for assaying large numbers of candidate regions or when resequencing pooled or heterogeneous samples. Next-generation sequencing is suited for large-scale discovery experiments, while CE with its high accuracy and unmatched data quality can be used to validate structural genetic variations.

We looked at *de novo* and targeted sequencing as two applications where each technology could be applied. In our analysis we consider sample preparation, and reagents, as well as key criteria that researchers consistently demand including accuracy, coverage, read length, quality values and ease of use.

#### P11.065

#### The Gen2Phen project: Collecting gene sequence variants and their phenotypic consequences in web-based LSDBs for Mendelian disorders

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The EU-funded Gen2Phen project aims to provide a holistic view of genotype-phenotype information. Gen2Phen facilitates the collection

of gene sequence variants and their phenotypic consequences by offering free support to establish and host Locus-Specific DataBases (LSDBs). To build, curate and share these gene variant databases, we have developed the Leiden Open-source Variation Database software (LOVD, <http://www.LOVD.nl>). As demonstrated during this presentation, after installation from CD, this free, open-source, platform-independent tool builds a basic database following current recommendations of the Human Genome Variation Society. The database manager can add any data field desired, e.g. to capture disease-specific phenotype information, deciding per field the input accepted and whether or not those data will be available for public display. LOVD supports several levels of data access (website visitor, submitter, curator, database manager), searching in and across data columns, custom design of direct links (to internet, intranet or even local-PC files), data exchange with central repositories (incl. NCBI, UCSC), automatic mutation nomenclature error checking using Mutalyzer and data storage on variants in different genes found in one patient. LOVD allows searching of data in non-public records, returning the number of hits and the option to ask for more information. Currently, LOVD is used to curate >260 LSDBs world-wide, with the Leiden server hosting >120 and data collected for ~60,000 variants from >30,000 patients, the largest series covering gene variants in relation to neuromuscular disorders.

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#### P11.066

#### Investigation the methylation status of promoter in JMJD1A of oligoazoospermia patients

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#### Introduction:

CpG islands in or near promoter region of many genes are subject to methylation which can have impact on gene expression. Thus, hypermethylation of promoter regions are known to be a cause of gene expression silencing and pathogenic in many genetic disorders. *JMJD1A* is a crucial gene for the final step of spermatogenesis. There are evidences that this gene directly controls expression of several genes required for DNA packaging in sperm cells and defects in this gene could be the cause for some cases of male infertility. In this study we investigated methylation pattern in *JMJD1A* promoter region in patients with unknown infertility.

#### Methods and materials:

In this study we prepared testicular biopsy from Iranian azoospermic infertile men for investigation of methylation status of 5' region of *JMJD1A* gene. Tissue sample of 5 obstructive azoospermic patients were used as normal controls. MS-PCR (Methylation Specific PCR) was set up to study methylation status in the promoter region.

#### Results and discussion:

Patients with non-obstructive azoospermia and infertility showed different pattern of methylation compared to normal controls. In brief, 100% of controls showed only unmethylated allele. In patient group, 3 patients (10%) showed only methylated allele. 2 patients (7%) showed both methylated and unmethylated alleles. The rest showed only unmethylated allele. This is the first evidence of involvement of epigenetic changes in *JMJD1A* promoter region in male infertility.

#### P11.067

#### Identification of functional SNP and putative PPRE sites in promoter of human malonyl-CoA carboxylase gene (MLYCD)

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Malonyl-CoA decarboxylase (MCD) catalyses the degradation of malonyl-CoA to acetyl-CoA. Malonyl-CoA is an intermediate in fatty acid synthesis and a potent inhibitor of carnitine palmitoyltransferase 1 (CPT1). CPT1 transfers long-chain fatty acyl-CoA (LCFA-CoA) to the mitochondria for  $\beta$ -oxidation. Reduced activity of MCD would lead to elevated LCFA-CoA in the hypothalamus, which signals energy surfeit and leads to inhibition of feeding. We proposed that genetic variation influencing expression of the MCD gene (MLYCD) could influence body weight through an effect on energy intake. It has been established that

peroxisomal-proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) activates transcription of rat hepatic MCD via two of the three PPRE sites identified (PPRE2 and PPRE3). We have identified the putative PPRE sites in human by alignment with the rat sequence. Promoter deletion analysis in HepG2 cells with overexpression of PPAR $\alpha$ /RXR $\alpha$  (retinoid X receptor  $\alpha$ ) showed that PPRE1 and PPRE3 were functional in human. We also studied the functional impact of the closest single nucleotide polymorphism (SNP) -379bp C/G (rs880088) to the putative PPRE sites in HepG2 hepatocytes and GT1-7 hypothalamic neurons in transient transfection studies. No significant difference in promoter activity was found between the alleles in HepG2 but the -379 G allele showed 2.1 times greater activity than -379 C allele in GT1-7 cells. Electrophoretic mobility shift assays (EMSA) revealed that the -379 G allele binds to nuclear protein(s) only from GT1-7 cells. In conclusion, the data suggest the MLYCD -379 C/G polymorphism is a regulatory SNP that affects promoter activity in GT1-7 cells.

#### P11.068

#### Loss of paternal methylation affecting the *MEG3* locus located on chromosome 14q32.2 imprinted region in a girl with maternal upd-like phenotype

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We report the case of a 8-year old girl seen in the orthopaedic department for scoliosis. She is the third child of non consanguineous healthy parents. She was born after an uneventful pregnancy (BW : 2.330kg, BH : 47.5cm, BHC : 32 cm, Apgar score 10,10). Neonatal feeding difficulties and moderate axial hypotonia were noted. Motor milestones and speech were delayed. From the age of 2 years, she began to put on weight and developed obesity. At 8 years 2/12, parameters are 38.4kg (+4SD), 136.5cm (+2.2SD), BMI 20.6; Tanner stages : S1A1P2. She presents a 40° dorsal scoliosis, rather short hands and feet, no dysmorphic facial features and no mental retardation. Cerebral MRI was normal. Karyotype is 46, XX, ish15q11-13(SNRPX2), and Array-CGH did not detect any microdeletion or microduplication.

As maternal uniparental disomy for chromosome 14 (maternal UPD 14) was suspected, microsatellite studies and methylation analysis of Maternally Expressed Gene 3 (*MEG3*) located in the 14q32.2 region were performed. Maternal UPD 14 was excluded by showing biparental inheritance of microsatellite markers. Methylation of the *MEG3* locus showed loss of paternal methylation with normal profil methylation of both parents.

Yet very few patients with exclusively maternal methylation profil of the *MEG3* locus have been described, but notably clinical features seemed to be grossly similar in epimutation patients and maternal UPD 14 patients.

#### P11.069

#### Both translation start sites in the human *MCT8* gene are used to produce two *MCT8* protein isoforms *in vitro*

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Mutations in *MCT8* (*SLC16A2*) are associated with severe X-linked psychomotor retardation and elevated serum T3 levels, also known as Allan-Herndon-Dudley syndrome. *MCT8* is an important thyroid hormone transporter, especially in the brain. In contrast to the mouse, the human *MCT8* gene contains two possible translation start sites (TLSs), giving rise to proteins of 613 (MCT8L) or 539 (MCT8S) amino acids. Studies in human liver revealed the presence of mRNA species containing both TLSs. Also, we described last year a patient with an insertion of 2 amino acids (p.G41\_S42 dup) located between the two TLSs in *MCT8*. In addition to short *MCT8* cDNA containing only the 2nd TLS, we cloned long *MCT8* cDNA with both TLSs, inserted the G41\_S42 duplication or mutated the second TLS (M75A, M75L) to prevent the synthesis of MCT8S. Western blotting showed that transfection with long *MCT8* cDNA results in bands of 61 and 69 kDa, representing MCT8S

and MCT8L. Transfection of long *MCT8\_M75L* or *MCT8\_M75A* cDNA resulted only in the production of MCT8L. Transport studies showed that uptake of thyroid hormone in cells expressing MCT8L was lower than in cells expressing MCT8S. However, the G41\_S42 duplication in the MCT8 protein did not change thyroid hormone transport activity, surprisingly in contrast to the studied human *MCT8* mutations so far. We found that both TLSs in *MCT8* are used to produce two isoforms which are both capable of transporting thyroid hormones *in vitro*. It is yet unknown whether these isoforms are differentially expressed in human tissues.

#### P11.070

#### Whole Genome Amplification of single cell: application in forensic SNP profiling.

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The scarcity of genomic DNA can be limiting factor in several fields of genetic research such as forensic, prenatal or preimplantation diagnosis. Multiple displacement amplification (MDA) is a whole genome amplification technique developed to amplify a limited DNA sample non-specifically to generate a new sample that is indistinguishable from the original but has a higher DNA concentration.

In this work we evaluated the applicability of MDA for forensic purposes. We amplified DNA, extracted from 30 single amniocyte cells and 10 single lymphocytes and typed a 36-STR panel. Single cell MDA DNA was typed twice for the same set of SNPs to evaluate the reproducibility of results. We performed a total of 2160 typing reactions observing a positive typing rate in amplified DNA (99,65%) and 97,87% of concordance rate between amplified vs control (genomic) DNA. The absence of perfect concordance rate revealed the failure of amplification of alleles in heterozygous samples (ADO). We also performed traditional STR-based profiling on single cell amplified DNA obtaining a higher ADO rate. These results suggest that MDA should be considered as a suitable option for SNP typing in challenging forensic casework.

#### Acknowledgements

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#### P11.071

#### Multiplexed DNA methylation testing for efficient tumor marker definition and microarray validation

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Here we present the design principle and performance of a combined multiplex-PCR and microarray hybridization technique for multiplexed methylation testing.

Targeting 323 published DNA regions hypermethylated in several neoplasias, methylation analysis is performed via methylation dependent restriction enzyme digestion of 500ng of starting DNA. DNA is amplified within 16 multiplex PCRs covering 360 different amplicons and detected via microarray hybridization. After PCR amplicons are pooled and positives are detected using streptavidin-Cy3 via microarray hybridization. Although the melting temperature of CpG rich DNA is very high, primer and probe-design as well hybridization conditions have been optimized, thus this assay enables multiplexed methylation testing of human samples. The assay has been designed that 24 samples x 360 reactions can be run in parallel on a single 384 well PCR plate. Several tumor entities have been tested using the assay. Chip-data derived from the multiplexed assay enabled classification and class prediction and defined candidate-markers with potential diagnostic application.

#### P11.072

#### Expression profiles of small RNAs from various tissues generated by SOLiD™ sequencing

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The combination of the SOLiD™ Small RNA Expression Kit (SREK) with the SOLiD Sequencing System presents a unique opportunity to study miRNA expression in a way not previously possible. We bar-coded and sequenced small RNA libraries from ten different human tissues to saturating levels of detection; generating up to 200 million tags. Comparing both independent sequencing runs and libraries indicates the system is highly reproducible and has up to 6 logs dynamic range. To analyze the quantitative ability of this approach we compared tag count data to real-time PCR assays generated using TaqMan miRNA low density arrays. Fold-change comparisons between platforms show Pearson correlation values of 0.95. Detailed analysis indicates a far greater repertoire of miRNA variants, or 'isomirs', than previously observed suggesting a much broader range of mRNA targets for miRNA-mediated regulation. Using this approach we have identified hundreds of potentially novel sequence tags. We chose a subset of these novel transcripts and designed custom TaqMan miRNA assays to validate them by real-time PCR analysis. We are able to demonstrate both the presence and expression profile of >50% of the novel sequences, most of which are present at relatively low levels in the ten tissues. Interestingly, we failed to detect nearly all of these novel targets using conventional northern blotting highlighting the need for qPCR sensitivity for validation purposes. This human miRNA expression atlas provides a unique opportunity to understand the sequence complexity and identity of small noncoding RNAs present in a variety of human tissues.

#### P11.073

#### MicroRNA target prediction by expression analysis of host genes

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MicroRNAs (miRNAs) are small noncoding RNAs that control gene expression by inducing RNA cleavage or translational inhibition. Most human miRNAs are intragenic and are transcribed as part of their host-ing transcription units.

We hypothesized that the expression profiles of miRNA host genes and of their targets are inversely correlated and devised a novel procedure, HOCTAR (host gene oppositely correlated targets), which ranks predicted miRNA target genes based on their anti-correlated expression behavior relative to their respective miRNA host genes. HOCTAR is the first tool for systematic miRNA target prediction that utilizes the same set of microarray experiments to monitor the expression of both miRNAs (through their host genes) and candidate targets. We applied the procedure to 178 human intragenic miRNAs and found that it performs better than currently available prediction softwares in pinpointing previously validated miRNA targets. The high-scoring HOCTAR predicted targets were enriched in gene ontology categories, which were consistent with previously published data, as in the case of miR-106b and miR-93. By means of overexpression and loss-of-function assays, we also demonstrated that HOCTAR is efficient in predicting novel miRNA targets and we identified, by microarray and qRT-PCR procedures, 34 and 28 novel targets for miR-26b and miR-98, respectively. Overall, we believe that the use of HOCTAR significantly reduces the number of candidate miRNA targets to be tested compared to the procedures based solely on target sequence recognition. Finally, our data further confirm that miRNAs have a significant impact on the mRNA levels of most of their targets.

**P11.075****Experimental and critical assessment of six methodological approaches to quantify heteroplasmy of mitochondrial mutations**

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Mitochondrial DNA (mtDNA) mutations have an important effect on the development of many genetic diseases, and are believed to play a role in aging and cancer. Most mammalian cells contain hundreds of mitochondria and each mitochondrion is endowed with several copies of mtDNA. However, an individual's mitochondrial asset may not always be uniform. After an mtDNA variation arises, it may be maintained, lost or amplified to different levels. As a result, cells and tissues, but also single mitochondria, may harbor both wild-type and mutant mtDNA, a condition known as heteroplasmy. In order for an mtDNA mutation to show a phenotypic effect, a certain threshold of heteroplasmy must be reached. Therefore, it is extremely important not only to qualitatively detect a mutation but also to provide an adequate and efficient quantitative analysis of heteroplasmy. Here we compare six different methodologies for their capacity to evaluate, capture and quantify different heteroplasmy levels. We also considered the issue of the potential bias introduced by preferential amplification during polymerase chain reaction (PCR). The mutation we chose to investigate is an insertion of a single base in ND1 gene described by Bonora et al (Cancer Res, 2007). Cloning and sequencing was set as the golden standard and the comparison was made between the following methods: fluorescent PCR, denaturation high performance liquid chromatography (DHPLC), quantitative real-time PCR (qRT-PCR), high resolution melting analysis (HRM) and 454 pyrosequencing. The final results will be presented.

**P11.077****In-lane Normalization Improves the Analysis of MLPA Data Obtained from Capillary Electrophoresis Systems**

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Multiplex ligation-dependent probe amplification (MLPA®, MRC-Holland) is an OLA-PCR- (oligonucleotide ligation assay PCR) based method used predominately for detecting copy number changes, such as whole exon deletions and duplications, in gDNA. Typically, MLPA reactions are analyzed on capillary electrophoresis (CE) instruments and specialized secondary analysis software is used to normalize the data and to calculate probe ratios to determine the presence of duplications or deletions. Here we describe the use of a new in-lane normalization reagent that is added to MLPA sample reactions prior to CE analysis. The data collection software on a newly developed CE instrument calculates a normalization factor that can then be applied to the raw MLPA data by the GeneMapper® v4.1 secondary analysis software. To demonstrate the benefits of in-lane normalization to MLPA data, gDNA samples for Pendred syndrome patients were analyzed using an MLPA kit (P280 Pendred-SLC26A4, MRC-Holland) designed to interrogate the causative gene. Pendred syndrome, the most common syndromal form of deafness, is an autosomal recessive disorder associated with developmental abnormalities of the cochlea, sensorineural hearing loss, and diffuse thyroid enlargement. Pendred syndrome results from mutations in the Solute Carrier Family 26, Member 4 (SLC26A4) gene. The MLPA multiplex kit consists of probes for each of the 21 exons of SLC26A4 in addition to two mutation-specific probes. We have found that in-lane normalization significantly improves the reproducibility and robustness of MLPA data analysis when using GeneMapper v4.1 software.

**P11.078****Generation of CreER-T2 transgenic mouse lines for temporal and cell type specific conditional gene inactivation: a tool for analysis of pathomechanisms in mouse models of monogenic diseases**

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The generation of mouse mutants by conventional knock-out shows two major limitations: (i) gene disruption often results in lethal phenotypes (ii) it does not allow site specific and time controlled inactivation of the gene of interest. Conditional knock-outs overcome these limitations. In the Cre-loxP system, the allele of interest is flanked by recognition sites for the Cre recombinase (loxP sites). When "floxed" mice are bred with transgenic mice expressing Cre in a tissue/cell-specific manner, the gene is knocked-out only in this tissue or cell. Temporal control is achieved using a ligand-activated recombinase, a fusion of the recombinase with a mutated ligand binding domain of the estrogen receptor (ER), which can only be activated by the synthetic ligand tamoxifen (Cre ERT2).

At ICS, we have generated about 50 Cre lines expected to express CreERT2 recombinase in different target tissues or cells. These include different neuronal populations, adipose tissue, different cell populations in the digestive tract, pancreas, muscle, bone, immune system, reproductive tract, skin ... Characterization of the efficacy and specificity of such lines is demanding, and we have devised a standardized flow-scheme (F. Gofflot et al.). Various lines are at different stages of characterization. We will provide the list of promoters and targeted tissues, and an update on their validation. These lines will be available to the research community and will be a powerful tool for the study of disease genes function, creation of disease models and to answer questions on the cell/organ autonomous or not character of pathological phenotypes.

**P11.079****New method and new tool for human mtDNA phylogeny reconstruction**

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Human mtDNA sequence variation is characterized by a high amount of homoplasmy which does not always allow unequivocal phylogeny reconstruction by conventional methods. We propose a novel maximum parsimony-based method for reconstruction of human mtDNA phylogeny. Briefly, the method consists of three successive stages: identification of potential homoplastic mutations; analysis of potential homoplastic mutations and identification of true homoplastic mutations; identification of back and parallel mutations. The method designed was implemented in mtPhyl. This software package allows rapid and comprehensive analysis of human complete mtDNA sequences. Apart from maximum parsimony phylogenetic tree reconstruction it performs different types of searches; analyzes mutation features; exports list of particular mtDNAs mutations into Excel table; defines mitochondrial haplotype; calculates coalescence time of clusters; estimates the effect of natural selection; makes reference list and downloads human mtDNA complete sequences from GenBank. mtPhyl represents a timely advance, since the advent of cheaper sequencing methods has generated an excess of sequence data, and there is an urgent need to perform their automatic analysis. Demo version of mtPhyl is available from the authors upon request and at <http://eltsov.org/mtphyl.aspx>.

**P11.080****Describing complex sequence variants by extending HGVS sequence variation nomenclature**

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New technologies allow rapid discovery of new sequence variants involving complex structural rearrangements. The description of these variants challenges the existing sequence variation nomenclature guidelines of the Human Genome Variation Society (HGVS, <http://www.hgvs.org/mutnomen>), which are mainly focused on simple variants. Here, we suggest extending the HGVS nomenclature guidelines with new description formats facilitating unambiguous and more detailed descriptions of most complex sequence variants. These include: 1) nested changes supporting descriptions of changes within inversions and duplications, 2) composite changes supporting concatenation of inserted sequences, 3) new duplication types describing changes in orientation. One advantage of this extension is that the differences and similarities between complex variants can be derived easily from the new descriptions. In addition, this extension is expected to provide sufficient flexibility and consistency to limit the proliferation of alterna-

tive interpretations giving rise to ambiguous descriptions of complex variants. The consistent specifications of the new description formats should allow easy implementation in sequence variant nomenclature checkers (e.g. Mutalyzer, <http://www.LOVD.nl/mutalyzer>). We are planning to extend the functionality of Mutalyzer to incorporate the latest version of the HGVS sequence variation nomenclature guidelines as part of the development of curatorial tools for Locus-Specific Databases (LSDBs) within the EU-funded Gen2Phen project (<http://www.gen2phen.org>). This project aims facilitate the collection of gene sequence variants and their phenotypic consequences by offering free support to establish and host LSDBs.

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#### P11.081

#### Validation of sensitivity and specificity of Tetraplet-Primed PCR (TP-PCR) in the molecular diagnosis of Myotonic Dystrophy type 2 (DM2)

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Myotonic Dystrophy type 2 (DM2, OMIM #602688) is a multisystemic degenerative disease caused by a tetranucleotide (CCTG)n expansion in the ZNF9 gene. Routine testing strategies require the use of Southern Blot or Long Range PCR but technical difficulties, due to the presence of very large expansions and wide somatic mosaicism, greatly reduce the sensitivity of present gold standard techniques. In order to simplify the diagnostic procedure for DM2, we developed a Tetraplet-Primed PCR (TP-PCR) for a fast discrimination of positive and negative patients. We validated the sensitivity of this technique analyzing 87 MD2 positive and 76 MD2 negative individuals, previously characterized by Long Range PCR. The specificity of the TP-PCR in the identification of (CCTG)n expanded alleles was evaluated testing 37 DM1 patients with different (CTG)n expansion. Our results show that TP-PCR is a fast, reliable and flexible technique, with a specificity and a sensitivity of almost 100%, with no positive or false negative results. We therefore consider that the use of this technique, in combination with the Short Range PCR, is sufficient to correctly establish the presence and the absence of ZNF9 exanded alleles in the molecular diagnosis of DM2.

#### P11.082

#### Comparision of Next Gen sequencing technologies and bioinformatic analysis tools - Case studies on different projects

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#### Objectives

Next Generation sequencers, e.g. Roche GS FLX and Illumina Genome Analyzer II, have been on the market for quite some time. A variety of different projects now reveal the pros and cons of each technology. The projects performed by GATC show that the use of one technology alone does not deliver the best results for all projects. Rather a combination of two or three technologies provides a more complete, cost-effective analysis. In addition to sequencing, bioinformatic analysis is critically important for gaining an in-depth understanding of the biological significance of the sequence data. The combination, analysis and visualisation of these data are key challenges to the successful application of the Next Generation sequencing technologies.

#### Methods and results

Having performed many projects, each with a different set of questions and goals. GATC presents sequencing data using single and paired end sequencing methods on different systems. Sequencing of mate pairs, especially using libraries with large inserts, helps tremendously to improve assemblies and alignments.

GATC uses a wide range of bioinformatic solutions for genome assembly and alignment, transcriptome analysis and other studies. We will present data comparing different bioinformatic tools which are available on the market.

#### Conclusion:

To ensure a successful sequencing project and to maximise the information obtained, it is necessary to choose the best Next Generation

technology or combination of technologies followed by bioinformatic analysis using a pipeline consisting of state-of-the-art analysis tools.

#### P11.083

#### Whole-genome human SNP detection using next-generation sequencing: accurate heterozygote detection at low coverage with diBayes algorithm.

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With the advent of next-generation sequencing, novel algorithms to detect SNPs using short-read data are needed. The dibase-coded oligonucleotides used by the Applied Biosystems SOLiD™ System allow SNPs to be distinguished from sequencing errors, facilitating sensitive and specific heterozygote detection at low coverage. We have developed diBayes, a Bayesian algorithm to detect SNPs in SOLiD reads. We apply diBayes to whole-genome sequencing of three HapMap humans. We identified over 6 million SNPs; ~80% in either African sample and 90% in the European are present in dbSNP, and the remainder are novel. We evaluated the sensitivity and specificity of diBayes, comparing SOLiD to HapMap genotypes; with a moderately aggressive setting of the algorithm, we call 85% of heterozygotes at 9x coverage, 95% of heterozygotes at 17x coverage, and more than 99% of heterozygotes at 23x coverage. The heterozygote false discovery rate for HapMap SNPs is  $8.5 \times 10^{-4}$ . Homozygous concordance is 0.9995. We annotated the damaging potential of non-synonymous SNPs (nsSNPs) with PolyPhen, and predicted 20% of nsSNPs to be damaging. There are significantly fewer damaging SNPs in homozygote than heterozygote state, consistent with the role of purifying selection. Using Pather ontology for annotation, we discovered that genes encoding transcription factors, ligases, growth factors, receptors, and are under-represented in the genes with damaging mutations. Further, GPCR genes involved in olfaction, and genes involved in immunity and defense are highly over-represented among genes with damaging mutations. Among OMIM alleles previously associated with human disease, very few are homozygous; none at highly penetrant Mendelian-disease loci.

#### P11.084

#### Targeted enrichment of selected genomic regions in preparation for Next Generation Sequencing

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Next Generation Sequencing technologies have revolutionized the search of genetic variants contributing to specific traits or disease. One significant challenge in exploiting their power effectively, however, is to isolate candidate regions from complex genomes specifically. To offer custom tailored services for the full experimental pipeline, we have established efficient protocols for oligo design and enrichment of such target regions on different array platforms, and for downstream use predominantly with the illumina Genome Analyzer II. Protocols for Roche and SOLiD sequencing systems have also been developed or are currently being established. Our enrichment success is monitored by quantitative RealTime PCR assays and classical sequencing of library subclones before loading the samples onto the sequencing instrument. We present exemplary results from our methods, and discuss the benefits of different strategies.

#### P11.085

#### Increased Read Length on the SOLiD™ Sequencing Platform

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The SOLiD™ Platform is an ultra-high throughput DNA sequencing system that utilizes sequential ligation of fluorescently labeled oligonucleotide probes. Improvements in the ligation biochemistry, sample preparation, and incorporation of an imaging buffer providing a higher signal to noise ratio have made it possible to routinely sequence 50 base reads from long fragment libraries, or 2x50 base reads from mate pair libraries with over 99.9% accuracy, generating over 20 gigabases

of mappable sequence data per run. Currently, novel ligation protocols have been developed to support increased read lengths of 75 bases to 100 bases, as well as reverse ligations to facilitate paired end reads. These longer read lengths increase throughput per run, facilitate resequencing efforts of large genomes, and aid in the identification of SNPs, indels, and other structural variations. Longer reads also lend themselves to novel applications of SOLiD™ such as RNA expression analysis and *de novo* sequencing.

#### P11.086

##### Targeted sequence enrichment for the SOLiD™ System

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The SOLiD™ system can acquire several gigabases of sequence within a single run, allowing for accurate resequencing of large genomes. The ultra high throughput of the SOLiD™ system also facilitates deeper sequencing of targeted genomic regions of interest for specific applications. Methods to enrich DNA in targeted regions include hybridization with custom oligonucleotides in-solution or on microarrays. For small-scale studies, microarray enrichment is more cost-effective, while in-solution enrichment is preferred for larger studies due to its scalability and potential for automation. Here, we report coupling of both enrichment techniques with SOLiD™ sequencing, using Agilent HD-CGH microarrays and in-solution probes to extract target regions from Yoruba DNA. We present an optimized SOLiD™ workflow for both enrichment strategies and provide deep sequencing results from these methods. The workflow from enrichment to SOLiD™ sequencing is streamlined by library construction prior to probe hybridization. Post-enrichment material can be used directly for downstream steps including emulsion PCR and sequencing on the SOLiD™ system. We demonstrate that combining these hybridization-based enrichment methods with the SOLiD™ system platform provides useful solutions for targeted resequencing applications.

#### P11.087

##### Whole Genome Microarray and Real-Time PCR to detect genes involved in non-syndromic ascending aortic aneurysms

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The non-syndromic ascending aortic aneurysm is a complex disease that involves a lot of people. The media coat is principally involved in the disease. Aim of this study is to identify gene expression differences between aneurysmal and normal ascending aortic media coats. A total of 41 aneurysmal aortic samples (cases) and 22 aortic samples without aneurysm (controls), had been harvested from patients undergoing aneurysmectomy and heart transplantation, respectively. After separation of the three coats, RNA from the media coats has been extracted, amplified and labelled according to standard protocols. Competitive hybridization of 3 single RNA cases vs a pool of 10 RNA controls has been performed on microarray platforms, consisting of 21,329 70mer oligonucleotides. Differentially expressed genes are validated by Taqman Real Time PCR. From the microarray analysis the following eight differentially expressed genes have been selected: Decorin, Receptor-interacting serine-threonine kinase 3, Osteoblast specific factor 2, Period homolog 2, EPH receptor A8, Adaptor protein with pleckstrin homology and src homology 2 domains, Resistin, CD2 antigen. Presently, semiquantitative analysis has confirmed up-regulation of Period homolog 2, and down-regulation of Decorin genes. Both these genes are involved in the integrity of the aortic wall, and in extracellular matrix remodelling.

Further expression analyses will better indicate pathways involved in thoracic aortic aneurysms development.

#### P11.088

##### Improving signal strength consistency in capillary

##### electrophoresis for high resolution fragment sizing applications

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Capillary electrophoresis (CE) is an important technology used for many DNA fragment sizing applications. Researchers have reported that when the same DNA fragment is analyzed by CE, a certain degree of variation in signal strength may be observed across multiple CE instruments; within a single instrument among different capillaries; or among different runs from the same capillary. For applications that interrogate human polymorphisms associated with disease, such as short tandem repeats, loss of heterozygosity, or single nucleotide polymorphisms, minimal signal variation is desired. Quantitative analyses such as quantitative fluorescent PCR or gene expression studies would also be improved by reducing signal variation. We describe the different sources of variation and elucidate two methods (one that adjusts hardware and one that uses chemistry) to obtain more consistent signal across the three levels of variation on CE: from run to run, capillary to capillary, and instrument to instrument. In combination, these methods have been observed to provide a significant reduction in the range of peak heights obtained across several instruments. This methodology would be useful to a single researcher or a consortium of investigators with multiple instruments who require the most consistent and comparable results possible from every instrument for high resolution fragment sizing applications. We describe a workflow from sample preparation to software analysis to quickly and simply obtain consistent results.

#### P11.089

##### A high resolution comparison of human and mouse alternative splicing patterns

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The HAVANA group will manually annotate all protein-coding genes, transcripts and pseudogenes on the human reference genome assembly as part of the ENCODE project, providing a significant resource for genome-science and medicine. Manual annotation is advantageous in the classification of alternative splicing, a process to which the majority of human genes are subjected. Transcription and splicing are error-prone processes, however, and GenBank contains immature ESTs and mRNAs; the proportion of the transcriptome which represents functional splice variation is therefore unclear. Here, we compare our annotation of the splicing patterns of 310 human coding genes within the ENCODE pilot regions against their mouse orthologs. We (1) investigate the conservation of alternative splicing at the transcriptional and splice site level, (2) classify conserved and non-conserved variants into 41 categories of alternative spliceform structure, and (3) categorise each variant as potentially protein coding or nonsense-mediated decay inducing. Conserved and nonconserved alternative splice events are seen to display distinct - perhaps unexpected - enrichments for different structural categories as well as functional potential. In line with previous reports, we observe that conserved alternative splice junctions are frequently (not always) embedded within near identical blocks of genomic sequence; we can now compare the presence of such elements with our inferred functionality of the corresponding splicing events. Overall, manual annotation generates a significantly higher resolution dataset than available to previous studies, affording a deeper view into the function and evolution of the alternative splicing process.

All HAVANA annotation is presented in the Vega genome browser: <http://vega.sanger.ac.uk>.

**P11.090****Methylation changes in p53 pathway in non-small cell lung cancer and their potential association with TP53 mutations**

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Methylation changes are common and relatively stable in various types of cancer. Mutations in TP53 may be present in up to 50% of non-small cell lung cancer (NSCLC) cases. It is known, that virtually all naturally occurring mutations in TP53 gene reduce the transactivating ability of p53 protein.

We have studied methylation of transcriptional start sites of wild-type p53 target genes in six NSCLC cell lines, two (A549, H292) with wild-type TP53 gene and four (H23, H520, H1703, H1299) with different TP53 mutations using combined bisulfite-restriction analysis. Six normal lung control samples were analyzed. For finding associations between methylation and expression repression, we carried out 5-aza-2'-deoxycytidine treatment. The analysis revealed presence of methylation in nine genes, but all the cell lines had a somewhat different set of methylated genes.

The number of hypermethylated genes was higher in cell lines with TP53 mutations. Methylation patterns of p53 transcriptional target genes were compared to the corresponding expression profiles. Hierarchical cluster analysis of the methylated genes showed their correlation with TP53 mutation status. Normal lung tissue controls with no TP53 mutations found by sequencing, clustered together with the wild-type TP53 gene cell lines.

5-aza-2'-deoxycytidine treatment caused decrease in the methylation of several analyzed genes and also induced a mild degree of apoptosis in the NSCLC cell lines with TP53 mutations.

**P11.091****SERPINA1 identifies papillary thyroid carcinoma across four distinct microarray studies and in independent validation data**

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**Background:** Several DNA microarray based expression classifiers for the different clinically relevant thyroid tumor entities have been described over the past few years. However, reproducibility of these markers is generally low, mainly due to study biases, small sample sizes and the highly multivariate nature of microarrays.

**Methods:** Therefore we adopted a meta analysis approach for four publicly available microarray datasets on thyroid carcinoma. Data integration to remove study specific bias was done using Distance Weighted Discrimination (DWD) and for feature selection and classification a nearest shrunken centroid algorithm (PAM - Prediction Analysis for Microarray) was used. Results were validated using independent Microarray and RTPCR datasets.

**Results:** Meta-analysis identified a one-gene classifier (SERPINA1) for papillary thyroid carcinoma. In Meta-analysis, identification of papillary thyroid nodules versus benign thyroid was achieved with 99% accuracy (97.9% weighted average accuracy in study crossvalidation). In the independent microarray validation data, which consisted of all major thyroid tumor entities, SERPINA1 correctly classifies 100% of nodules into one of the two classes PTC and non-PTC.

**Conclusions:** These results show that new insights can be gained using existing data and indicate a huge potential for future diagnostic applications.

**P11.092****High throughput phenotypic screens in Parkinson's Disease**

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Parkinson (PD) is a neurodegenerative disease with a huge socio-economic burden. In PD years of slowly progressing neurodegeneration set the stage for devastating clinical phases. Current treatments are only symptomatic which ultimately result in debilitating side effects thus there is an urgent need to develop therapeutics which stop and reverse disease progression. 13 loci have been implicated in the pathogenesis of PD, with only 7 of the genes underlying the loci hav-

ing been identified. These genes have suggested that disruption of a myriad of molecular pathways (e.g. ubiquitin and proteasome, mitochondrial function and protein misfolding) can lead to the degeneration of the substantia nigra. However it remains unclear how mutation of these genes and disruption of these pathways lead to PD.

To address these issues, we have developed a high throughput and high content screening facility using siRNA to modulate the expression of every gene in the genome and determine their effect in a range of cellular assays related to PD. Several assays have been established to measure several processes which have been implicated in PD pathogenesis such as  $\alpha$ -synuclein aggregation and phosphorylation, DJ-1 related functions (apoptosis, translocation and oxidation). The goal is to construct a detailed molecular pathway of the proteins that are involved in the function of genes mutated in PD. This will provide a greater understanding of the molecular pathways involved in PD and if any of these can be modulated or exploited for their therapeutic potential.

**P11.093****Putative interactors of Pax6 indicate novel molecular cascade of its function**

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**Objective:** The Pax6 (a transcriptional regulator) is critical for brain and eyes development. The mutation in Pax6 leads to severe brain and eyes anomalies. Phenotypes are of variable penetrance and expressivity. It is presumed to be influenced by protein-protein interaction (matricellular proteins/TGIF/TGF/Neurotrophins)

**Methodology:** Models of Pax6 interacting proteins were analysed through on-line servers (STRING and PIP). The interacting proteins of Pax6 were also explored through co-immunoprecipitation and co-localization

**Significant Results:** Novel interaction of Pax6 with SPARC (secreted protein, acidic and rich in cysteine, a matricellular glycoprotein of 43kDa) was observed. The immunoreactive bands with Ras and p53 were also detected in the sample of brain immunoprecipitated with anti-Pax6

**Conclusions:** The observations elucidate novel interactors in the cascade or hierarchy of Pax6 or SPARC functions. The putative interactors also provide link to Akt and TGF-beta pathways through SPARC, Ras and p53. The preliminary observations are useful in exploring molecular basis of nervous system function

**P11.094****Rapid single-molecule haplotyping in patients with sickle cell disease**

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Genome-wide association studies have provided many new genes influencing disease risk and severity as well as biometric traits. Identifying causal variants at these new loci has become a bottleneck and poses a challenge for technology: how to extract useful information from a focused genomic region in large numbers of samples. High-density SNP genotyping provides a point-by-point survey of the region of association, but additional information could be gained from the sequence of SNP alleles on each parental chromosome (phase), i.e. haplotypes present in each study subject.

We have used an integrated fluidic circuit (IFC) system from Fluidigm Corporation (the digital array) to analyze multiple SNP loci from individual molecules of sample DNA and to rapidly obtain haplotype information over several kb of target sequence. Using this approach, we were able to phase double heterozygous SNP genotypes over distances of 0.7, 1.2, and 5.2 kb. We have studied three SNPs (rs9399137, rs9402685 and rs11759553) at a locus on chromosome 6q23 (HBS1L-MYB) that modifies clinical severity in sickle cell disease, a monogenic blood disorder due to a mutation of the  $\beta$  hemoglobin chain. We detected four common haplotypes (with 46%, 21%, 14% and 13% frequency) and a single infrequent one (5.5%), which is associated with milder disease. We plan to extend phasing to a fragment of 24 kb to help identifying the causative DNA variants at this locus.

**P11.095****New targets of Semax (N-terminal ACTH fragment analog) action on gene expression: temporal and spatial dynamics**

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Semax is a synthetic peptide which consists of the N-terminal adrenocorticotropic hormone fragment (4-7) and C-terminal Pro-Gly-Pro peptide. Semax is used in the treatment of human neurological diseases. Our recent findings revealed that single intranasal Semax administration results in rapid and specific for different rat brain structures *Bdnf* and *Ngf* genes expression changes. So we investigate dynamics of expression of some genes of *Wnt*, *NF-kB*, *Jak-Stat* and retinoic acid signal transduction pathways (*Pparg*, *Nos2*, *Mig*, *Rbp1*). As we can see all genes under study significantly changed their expression levels 40' after Semax administration and these effects were still observed 24 hours after the peptide treatment. Both hippocampus and frontal cortex tissues demonstrated gene expression changes. Particularly in the frontal cortex expression dynamics of *Mig* and *Nos2* were analogous most part of the experiment. In the hippocampus the expression similarities were observed for *Pparg* and *Rbp1* genes and for *Nos2* and *Mig* genes in pairs from 3 till 24 hours after Semax treatment. Comparing hippocampus and frontal cortex we can see that all gene expression in the first structure deeply decreased 24 hours after the peptide administration. We can see that gene expression reaction on Semax application in hippocampus is more continuous then investigated time period. Gene expression in the frontal cortex on the contrary mostly returned to its basic level to the end time point of the experiment. It could be evidence of the more distant changes in the hippocampus compared with frontal cortex under Semax administration.

**P11.096****Preaxial Polydactyly: Two novel mutations in sonic hedgehog long-range regulator and one negative family**

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The limb-specific long-range cis-regulator of Sonic Hedgehog (SHH), called ZRS (ZPA Regulating Sequence) is a highly conserved regulatory sequence 1Mb away from SHH. This regulator allows the patterning of SHH expression to the posterior limb bud.

Point mutations or complete duplications of the ZRS have been shown to cause ectopic expression of SHH in the anterior limb bud, leading to autosomic dominant preaxial polydactyly (PPD2, MIM #174500) in humans, mice, cats and dogs.

We present here our analysis of the SHH locus in three large French families with isolated PPD2.

Two families have full-penetrant point mutations in the ZRS, 297G>A, 334T>G (according to the original numbering by Lettice). Bioinformatic analysis revealed that these point mutations are located in highly conserved predicted transcription factors (TFs) binding sites for SOX9 and PAX3. Those TFs are simultaneously expressed with SHH in the mouse developing limb bud at the time of digit patterning, and have been respectively involved in polydactyly and limb development defects.

Analysis of the third family revealed possible linkage with SHH locus, but no point mutation or copy-number variation of the ZRS could be detected by sequencing and QPCR. CGH-array revealed no anomaly at SHH locus or in any other genomic region. We then sequenced five other highly conserved candidate regions around SHH that contained conserved TFs binding sites concerning TFs of interest: no mutation was detected. We hypothesize that another nearby regulatory sequence or an undetected position effect between ZRS and SHH could be responsible for this familial case.

**P11.097****The Sri Lankan Genome Variation Database**

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Sri Lankan Genome Variation Database (SLGVD) is a database of genetic variations found in Sinhalese, Sri Lankan Tamils and Moors, the three major ethnic groups in the Sri Lankan population. Studies of variations in genes among different groups of individuals in the Sri Lankan population have grown rapidly during last few years. These

studies generated large amount of genetic data which is important to study the occurrences of diseases that differ across ethnic groups. There is therefore a need for a central repository of this data. The SLGVD was created to fulfill this void. This offers a web based access to genetic variation information of Sri Lankan people. It would also be an important informatics tool for both research and clinical purposes to retrieve and deposit human variation data. The database was designed confirming to guidelines issued by the Human Genome Variation Society (HGVS). The variation data catalogued in SLGVD were derived from research performed by Sri Lankan Scientists. Addition to variation data each variation links with the relevant entries of Online Mendelian Inheritance in Man (OMIM), SNP and Genbank databases at National Center of Biotechnology Institute (NCBI). For each variation, genotype and allele frequencies of different ethnic groups are represented in numerical and graphical format. SLGVD can be publicly accessed from <http://hgucolombo.org/default.aspx>.

**P11.098****Protein Thermal Shift Assay Using Applied Biosystems Real Time PCR Instruments**

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The Protein Thermal Shift Assay (TSA) is a rapid and sensitive tool for monitoring protein thermostability, aiding in the identification of optimal conditions or conformations/sequences that favour protein stability, including the investigation of protein-ligand interactions. TSA is based on temperature-induced protein denaturation, monitored using an environmentally sensitive dye, such as SYPRO Orange, that is naturally quenched in a stable, aqueous solution. As the temperature increases, exposed hydrophobic regions will bind the SYPRO Orange dye, leading to a proportional increase in fluorescence that is measured using a real-time PCR instrument. The inflection points of the resulting fluorescence/temperature plot are used to compare different test conditions, and the extent of the observed temperature shift is considered to be representative of the stability of the protein in certain solution conditions. TSA data have been obtained from the whole range of Applied Biosystems Real Time PCR Systems; including the 7900 HT, 7500 Fast and StepOnePlus™ Real Time PCR Instruments. TSA has wide-ranging applications, including high-throughput screening for unknown ligands and protein inhibitors, protein-substrate interactions, and concentration-dependent stabilisation conditions. In addition, TSA benefits protein crystallography studies, for determining the concentration of a compound that will provide maximal stability in order for a protein to reach maximum occupancy. The benefits of performing a TSA with an Applied Biosystems Real Time PCR System include the flexibility of run-method programs, catering for a range of data resolution requirements and in the use of small reaction volumes, providing fast and accurate results with only a few µg of protein.

**P11.099****Whole-gene expression system by RMCE-mediated integration of PAC clones**

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Systems to express whole-genes in cell lines would be highly desirable in order to overcome the limitations of current reporter-based assays in the functional assessment of mutations within intronic or regulatory regions. Here we achieved whole-gene over-expression of the tyrosine phosphatase LYP, a critical regulator of signaling through the T cell receptor, encoded by a major autoimmunity gene in Jurkat T cells by single-copy RMCE-mediated genomic integration of a PAC clone. The PAC clone was recombined in order to tag exogenous *PTPN22* and to provide a sortable marker of PAC integration. Our system can be useful for the study of gene regulation and in functional genetics of polymorphisms and extended haplotypes of *PTPN22* and other candidate autoimmunity genes.

**P11.100****Expression Profiling of both miRNAs and mRNA targets using a novel nanofluidic real-time qPCR technology**

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Previously, profiling both microRNAs and their mRNA targets was difficult, time consuming, and involved extensive post-profiling validation. Our system allows for easy, rapid and inexpensive quantitation of both miRNA and mRNA targets with no need for validation. The reliability of real-time qPCR, the flexibility of an array format and novel nanofluidics provide the backbone of this elegant system. Using the OpenArray system, we profiled RNA from a panel of human adult tissue types for a set of well characterized miRNAs. In our second study, total RNA was obtained from normal tissues and tumor tissues, reverse-transcribed and loaded on plates preformatted with primer pairs against hundreds of genes that have been found to be differentially expressed in cancer. This pre-validated qPCR panel covers disparate, but linked pathways such as DNA repair, angiogenesis, cell adhesion and cell cycle. Using this nanofluidic system we were able to identify genes that consistently changed expression across cancer types as well as genes differentially expressed in specific cancers. The OpenArray system allowed us to concurrently chart both the modulator and the modulated. An analysis of the data provides us with a rich view of the complexities of gene expression and further validated our technique. The simple power of this technique is that the researcher does not need to further validate their results with qPCR; they have simultaneously performed both the discovery and validation phases.

**P11.101****Reference materials for genetic diagnostics and HLA-typing**

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The National Institute for Biological Standards and Control (NIBSC) in collaboration with diagnostic laboratories have developed a programme to generate genetic reference materials (GRMs) as WHO International Standards and CE-marked In Vitro Diagnostic Controls. The materials are used for genetic diagnostics, both for genetic disease and tissue typing (HLA-typing), and are typically positive control DNA samples of known genotype which serve to verify assay, operator and data tracking performance. The genetic material is sourced from cell lines established from primary lymphocytes to produce quality-assured cell banks.

CE-marked HLA-typing panels are produced within the ISO13485 quality system. The HLA-A genotyping panel comprises 24 genomic DNA samples and provides single-use positive controls in DNA-based low resolution HLA-A typing for many common alleles in the UK Caucasian population. The panel has been awarded the CE-mark and is expected to be available in 2009. A HLA-DRB1 panel of 40 genomic DNA samples is currently in production.

WHO International Standards of genomic DNAs have been produced for Factor V Leiden, Prothrombin G20210A, Haemophilia A intron 22 inversion and Fragile X syndrome. A genomic DNA GRM for Prader Willi and Angelman syndromes will be submitted to the WHO or another international organisation in 2009. GRMs for other genetic disorders, including a panel for hereditary non-polyposis colorectal cancer with genomic DNA deletion samples are planned. NIBSC aims to address the rapid increase in the numbers of tests performed and the development of new technologies by the provision of GRMs as a key tool in successful genetic diagnostics.

**P11.102****The Locus Reference Genomic (LRG) DNA sequence format**

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A crucial element of sequence variant nomenclature is the reference DNA sequence used to describe the variant. NCBI has established curated non-redundant reference sequences of genomes, transcripts, proteins and genes (RefSeq & RefSeqGene). However, these are not ideal for reporting variants in Locus-Specific Databases (LSDBs).

The primary limitation is that the reference DNA sequence might be revised over time, reflecting current knowledge, but failing to provide a

stable reference for inter-generational genetic diagnoses. Additionally, there may be several alternatively-spliced transcripts. Furthermore, legacy exon- and amino-acid-numbering systems may have arisen for particular genes and be in common use, even though they do not comply with current variant reporting standards.

To address these limitations, we have developed a new sequence format known as Locus Reference Genomic (LRG) that will have both locked and updatable sections.

The locked section will primarily comprise:

- The DNA sequence
- Coordinates of all identified exons
- Details of coding transcripts and their conceptual translation

The updatable section will primarily comprise:

- Coordinates and other information to map a LRG onto the current human genome build
- The exon-numbering scheme
- Details of legacy DNA reference sequences, RefSeq and RefSeqGene sequences
- Details of legacy exon- & amino-acid-numbering systems
- Chromosome number
- Known sequence variants
- Audit data for each feature

Details of LRGs, which are implemented in XML, accompanied by sample sequences can be found at <http://www.lrg-sequence.org>

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**P11.103****Dual luciferase reporter assay system: limitation of its interpretation**

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Genetic reporter systems are popular tools for studying many processes that take place in eukaryotic cells, e.g. transcriptional regulation, mRNA processing, etc. Usually, dual reporter enzymes, an experimental reporter and a normalizing standard, are used in one experiment to minimize an interassay variation caused by differences in transfection efficiency and cell viability. Consequently, the results are expressed as the reporter enzyme activity normalized to the activity of internal standard. Assuming that the same amount of reporter genes is always used in one experiment, the ratio of its expression (and thus ratio of enzyme activities) should be a constant. No correlation is supposed between the ratio of reporter activities and the number of transfected cells or the activity of the reporters. However, in our experiment, there is a correlation between the ratio of reporter activities (luminescence of different luciferases) and the experimental reporter activity. Using one mixture of reporter and standard gene constructs for transfection of different amounts of cells, we obtained ratios of luciferases activities strongly correlated to the firefly luciferase activity ( $r = 0.84$ ;  $p < 0.001$ ). This effect was specific for the particular construct since neither the construct with shorter insert of the same type nor the reporter gene-containing plasmid without any insert showed this effect. We speculate that some specific expression activator could induce such non-standard outcomes. These results clearly indicate that the reporter gene assays data should always be evaluated with care.

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**P11.104****Identifying Users and Contributors on the Biomedical Internet**

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A number of ostensibly separate initiatives have begun considering the risks, benefits, and practicalities of unambiguously identifying researchers as they use and contribute to biomedical data sources on the Internet. The GEN2PHEN project (<http://www.gen2phen.org>) is one such initiative, given its general aim of helping to unify human and model organism genetic variation databases towards increasingly holistic views into Genotype-To-Phenotype (G2P) information. More specifically, the GEN2PHEN project considers researcher identification to be an absolutely central part of how biomedical databasing, and

scientific reporting in general, needs to be developed.

At the heart of this lies the concept of a user-centric system for researcher identification - i.e., one or more 'ID systems' by which individuals can be unambiguously identified along with various types of information associated with them, and where the individual controls his/her online identity and how/where it is used. At present, key Web 2.0 Internet technologies which can underpin such a system (e.g., OpenID - a decentralized, open authentication protocol), are being widely adopted.

To advance this field, a community of key stakeholders (e.g., GEN2PHEN, P3G, HUGO, HVP) has been assembled and is continually growing. This group is exploring innovative ways to exploit this new Internet ecosystem to support research-related activities and services. A dedicated website is provided (via [www.gen2phen.org](http://www.gen2phen.org)) where issues are being discussed, and a workshop on the subject has been planned for May 2009.

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#### P11.105

#### Abundance and orientation bias of retroelements in mammalian genes

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In different eukaryotic genomes some transposable elements (TEs) located in introns of genes show significant bias with predominant antisense orientation of TEs. It was suggested that this bias can reflect negative selection against TEs in the orientation of coding strand. In mammalian genomes retroelements of L1 family are known to be strongly underrepresented in GC-rich gene rich regions but their abundance and orientation in introns of genes is poorly studied. Here we analyzed abundance and orientation of major families of retroelements in introns and promoters in eight large groups (>300 genes each) of coexpressed human and mouse genes. We found that human Alu and mouse B1 repeats are overrepresented in introns and upstream regions of all studied groups of genes with significant bias for their inverse orientation in groups of the widely expressed (housekeeping) genes. L1 elements are underrepresented in all studied groups of genes especially in the upstream regions and only ~30% of L1 is found in direct orientation. LTRs are also underrepresented in all groups of human and mouse genes with only 15-30% of their copies present in direct orientation suggesting strong selection pressures operating during evolution on retroelement content of promoters and introns of human genes. Purifying selection of LTRs, L1 elements and some Alu repeats in direct orientation is apparently a consequence of their negative effects on normal transcription and/or splicing. Accumulation of Alu and B1 in the housekeeping and some tissue-specific genes may be caused by positive selection of complex genome rearrangements facilitating transcription.

#### P11.106

#### MeCP2 gene point mutation analysis, gross deletions and X-chromosome inactivation in 200 girls with Rett syndrome and with variants of the disease in Russia.

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Rett syndrome (RTT) is a neurodevelopmental disorder inherited in an X-linked dominant manner affecting almost exclusively females. *MeCP2* gene (Xq28) is responsible for both classic and atypical cases of Rett syndrome. Mutation analysis of *MeCP2* gene was performed in 200 female patients with classic and variant phenotypes of RTT and with non-specific mental retardation by using SSCP analysis and sequencing of the coding region of the *MeCP2* gene. Prior to mutations screening we have excluded Angelman syndrome (methylation in 15q11-q13 region), Smith-Magenis syndrome (17p11.2 deletion) and CGG expansion of *FMR1* in patients with non-specific mental retardation.

More mutations in this study have been identified in 64% patients with

classical forms (half of these mutations were revealed in 8 hot spots of *MeCP2* gene) of Rett syndrome compared to atypical patients with mental retardation (28%).

Gross rearrangements of the *MECP2* gene, which are not detectable by sequencing or SSCP, have been identified using dosage assays including quantitative fluorescent PCR and real-time PCR. Large deletions were identified in 15% cases of our patients in whom no *MECP2* mutation had previously been detected by sequence analysis.

Variability of Rett phenotype has been partly attributed to an effect of X-chromosome inactivation. In the same cohort of patients skewed X-inactivation was detected in 10% RTT cases. Moreover, phenomenon of nonrandom X-inactivation (NXI) assessed by CAG polymorphism analysis of *AR* (*HUMARA*) gene was detected in mothers of RTT patients (38%). In these cases NXI was found in the absence of mutation and clinical features of diseases.

#### P11.108

#### Some points for the optimization of the semiquantitative multiplex PCR assay for detection of exon rearrangements in the *Parkin* gene

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Mutations in the *parkin* gene are responsible for a notable fraction of autosomal recessive early onset Parkinson's disease incidence worldwide. Exon rearrangements in the gene constitute an important class of the mutations. In our study we performed a commonly used semiquantitative multiplex PCR assay for detection of rearrangements of *Parkin* exons. The assay essentially compares relative amounts of template DNA in a PCR reaction, thus allowing identification of templates that deviate from the expected number in a diploid genome. In order to detect these rearrangements, several exons are PCR amplified simultaneously, allowing comparison of relative amplification of exons in different samples. The co-amplified exons serve as internal standards for quantification. The amount of PCR product is directly related to the number of template molecules as long as PCR is terminated within the exponential phase of amplification. Each PCR for a given combination of exons results in a typical pattern of peak heights for normal control DNA, thus producing reference ratios between the peaks. Because ratios are compared, extraneous factors are expected not to interfere with detection of rearrangements.

Our study resulted in identification of several exon deletions in the *Parkin* gene. We noted that for optimal detection of rearrangements, careful attention must be made to several factors: -all the fluorescently-labeled primers in a combination should be labeled with the same dye; -height of exon peaks reflecting extent of amplification should be very similar for the different exons (less than 10% difference); -quality of the DNA samples should be high.

#### P11.109

#### High coverage, low bias libraries for the SOLiD™ 3 System to support 2x50 mate pair libraries

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Paired end sequencing facilitates sequence assembly and broadens the application of next generation sequencing technologies. Library construction strategies that utilize type III restriction enzymes, such as EcoP15I and Mmel, generate mate-paired libraries with limited sequence tag length. As the read length of the SOLiD system improves, a library construction method that generates longer DNA mate pairs is necessary. We describe a paired end library construction method that uses nick translation activity of DNA polymerases and T7 exonuclease to generate DNA mate pairs. The length of the DNA mate pairs can be adjusted by controlling the temperature and time of the nick translation reaction. We have prepared multiple paired end libraries with tag lengths of 75-100bp using many complex genomes. These libraries have been extensively sequenced at 50 bp for each tag using SOLiD,

AB's next generation sequencing platform. We have determined several conditions that result in high coverage and low bias libraries. These libraries enable the ability to read 50 bases on each tag, allowing rapid and precise mapping of structural variations, including translocations, across the entire genome of complex organisms.

#### P11.110

#### Improving the quality of DNA libraries in the Next Generation Sequencing workflow

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Next-generation sequencing technologies play an important role in investigating complete cancer genomes and transcriptomes. To further increase productivity of this compelling technique, the quality of DNA libraries plays an important role. One important step in the Illumina sequencing workflow is the amplification of the generated libraries for determining the exact sequencing cluster concentration. The drawback of this step is that amplification artefacts and errors will be introduced into the target sequence.

An on-chip electrophoresis instrument has become a standard tool for implementing DNA library quality control and quantification in the Illumina workflow. The microfluidic device monitors the size and quantification of the amplified libraries and also helps to detect contaminating artefacts. With an optimized protocol and newly developed electrophoresis chemistry, the sensitivity could be increased by a factor of 20-30, down to the pg/μl concentration range. This improved detection sensitivity allows for the significant reduction of amplification cycles thereby reducing the target sequence error rate.

#### P11.111

#### mapreads: a tool that rapidly maps short reads to a genome

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Mapreads is a bioinformatics tool that is optimized to rapidly map short SOLiD (color space) or sequence reads to the genome. Mapreads indexes the genome for a specific word size (W=14/15 is default for mapreads), but unlike BLAST can use both continuous and discontinuous word patterns called "schemas". An example of a discontinuous schema for a 25-mer will be 7 matches in the beginning of the read followed by 11 characters followed by 7 matches (effective W=14). Only seven W=14 schemas are required to map a 25-mer with up to two mismatches (25-2) enabling much quicker searches than BLAST W=7. A new feature of mapreads allows to specify the number of mismatches for each schema and report "k-best" hits in the genome. A single base difference between a read and the reference leads to two color differences - so called valid-adjacent (VA) mismatches. Mapreads can count these as a single (VA=1) or two (VA=2) mismatches. In addition mapreads supports IUB codes in the reference sequence enabling to avoid "non-reference allele bias" when mapping short reads to known SNPs. We present results how mapping and false-positive / false-negative SNP-calling rates for human paired 25-mers depend on mapping parameters. We use both artificially generated color-reads with "spiked errors" and SOLiD sequences from a HapMap individual with known genotypes. Performance for mapping to human genome is presented. The source code of mapreads is available under GPL from <http://solidsoftwaretools.com/gf/project/mapreads/>

#### P11.112

#### Multiplex Sequencing on the SOLiD™ Platform with 10, 16, or 96 Barcodes

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The SOLiD™ DNA sequencing system utilizes stepwise ligation of oligonucleotide probes and enables high fidelity, high throughput sequencing. In order to maximize sequencing capacity and reduce workflow of sample preparation, a single sequencing run containing multiple biological samples is sometimes preferred. To this end, a multiplexing method with barcodes has been developed for the SOLiD™ platform.

Barcodes are unique 5-7 base sequences that are added at the 3' end of the template along with a barcode priming region. Sets of 10, 16, and 96 barcodes have been designed and can be assigned to up to 96 individual samples. Data presented shows the sequencing results of all three sets of barcodes, as well as the results of an alternative design of 20 barcodes. For all sets of barcode analysis, over 96% of matching sequenced tags contain a barcode. The multiplexing system coupled with SOLiD™'s ability to process two slides, accommodating two to sixteen depositions, enables researchers to sequence over a thousand patients or unique biological samples within a single run.

#### P11.113

#### The Detectable Genome: How much of the human genome is accessible to variant discovery by next-generation sequencing?

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The human genome is being vigorously sequenced in an effort to understand the extent of normal human variation as well as disease causing variants. This initiative brings with it the challenge of assessing the areas of the human genome that are accessible to variant detection. We illustrate the amount of the human genome that is covered with uniquely placed single tags and uniquely placed mate pairs and demonstrate how both larger insert sizes and read lengths increase the portion of the genome that is uniquely mappable by paired-end tags. We use various human genomes (NA18507 - 10x Yoruban male, NA19240 - 26x Yoruban female) sequenced with SOLiD™ sequencing to illustrate the amount of SNPs and indels that are detected at various levels of average sequence coverage. We also demonstrate the sequence and clone coverage needed to identify indels of any size between paired-end reads. We use libraries with an assortment of insert sizes to show that larger libraries increase the accessibility of the genome by spanning larger insertions. We show that the bisulfite converted human genome is less uniquely mappable than the normal human genome but significantly less signature is lost in color space than in base space. We also illustrate that a significant portion of large segmental duplications are accessible to sequence and clone coverage by paired-end reads. These principles are applicable to all next-generation sequencing platforms and are essential to comprehend the amount and location of variability in the human genome.

#### P11.114

#### Tissue-specific forkhead protein FOXA2 regulates SOX14 gene expression

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Sox14/SOX14 is a member of B2 sub-group of Sox/SOX gene super-family that functions as transcriptional repressor. Its expression is restricted to a limited population of neurons in the developing brain and spinal cord. In spinal cord explants, expression of Sox14 was found to be regulated by Sonic hedgehog (SHH). Foxa2 (previously named Hepatic nuclear factor-3-beta: HNF3β) displays a remarkable functional diversity and is involved in a wide variety of biological processes during development and adulthood. In the developing nervous system, Foxa2 can be detected in the floor plate of the spinal cord and in periventricular areas of the midbrain and diencephalons.

We have proceeded with investigation of transcriptional regulation of human SOX14 gene expression. Our gel-shift and super-shift experiments demonstrated that FOXA2 interacts directly with predicted binding site within the SOX14 enhancer region. Using mutated oligonucleotide probe we further confirmed specificity of the FOXA2 binding to the predicted site. Results obtained with Foxa2 over-expression in co-transfection experiments confirmed that SOX14 enhancer region possesses regulatory element activated by this protein in HepG2 and U87MG cells.

In conclusion, here we present the first evidence that transcription factor FOXA2 is involved in the up-regulation of human SOX14 expression in HepG2 and U87MG cells by direct interaction with the binding site within its enhancer region.

**P11.115****Expression of genes involved in self-renewal or lineage priming in mouse embryonic stem cells**

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Profiling gene expression in embryonic stem cells (ESCs) requires technology that allows the use of very small samples, yet has the capability to analyze the expression of many mRNAs simultaneously. A three step workflow including reverse transcription, multiplex pre-amplification, and singleplex PCR in TaqMan® Arrays was used. We demonstrate that multiplex preamplification-based TaqMan® real-time PCR can be used to profile gene expression in limited starting material or single cells. A total of 384 genes which are believed to be functionally associated with maintenance of the undifferentiated embryonic stem cell state were studied in mouse embryonic stem cells (mESCs), differentiated embryoid bodies (mEBs), and embryos. Three endogenous control genes, Ctnnb1, Actb and Gapd were used to confirm the uniformity of the PreAmp reaction and to normalize RNA input. Preamplification of starting cDNA molecules enables detection of low expressers from nanogram amounts of total RNA input and even single embryonic stem cells. A novel subset of differentially expressed mRNAs was established, which can be used as biomarkers to distinguish the pluripotent state from cells that have undergone differentiation. Preamplification makes it possible to profile hundreds of genes from limited starting material, including single cells, reproducibly and without amplification bias.

**P11.116****Exploiting The Full Potential Of Sequence Trace Files For Structural Variation**

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Structural variation and copy number variation (CNV) are recently recognized to be extensive in the human genome. Examples identified so far typically range in size from 5-200kb and encompass many genes which play fundamental roles in both disease and evolution. The impact of structural variation on genome function and disease is likely to be substantial.

Currently known structural variants have been identified by a range of technologies - both experimentally and in silico. However, these approaches are very inefficient at detecting rarer, shorter (<10kb) and lower similarity variants. The reduced costs of sequencing and new high-throughput technologies mean there has been an exponential growth in the raw primary data used to assemble genomes. These publicly available and extensive data will carry immensely detailed information about structural variation, but systems need to be provided to mine this knowledge.

We have therefore developed a dedicated computational tool that utilises raw sequence data from the world's total public dataset of trace files to reveal and allow visual exploration of small (and large) structural rearrangements.

Methods of analysis include; i) trace depth analysis, ii) discordant trace end alignment, iii) trace file source filtering, iv) similarity of match considerations - all linked to a powerful graphical browser with extensive tuning options and connections to many other forms of genome annotation.

This tool will benefit many research areas, such as primary genome polymorphism analysis, disease association studies, and investigations into the genomic mechanisms of cancer development.

**P11.117****Detection of Structural Variations Using SOLiD™ Mate Pair Sequencing Technology**

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Structural variations in the human genome - insertions, deletions, inversions and translocations - are important aspects of genetic variations that define individual genotypes and phenotypes. For example, chromosomal inversions are associated with Angelman Syndrome, Hemophilia A and other abnormalities. We used the SOLiD™ high-

throughput sequencing technology with paired-end tags to sequence three HAPMAP genomes NA18507 (Yoruba male, 6x), NA19240 (Yoruba female, 14x), and NA12878 (CEPH female, 12x).

We developed an analysis pipeline to detect the occurrence of structural variations in our samples compared to the reference genome. A deletion, an inversion or a translocation is defined by its two breakpoints while an insertion by a sole breakpoint. The supporting evidence for occurrence of breakpoints is scored for each base pair in the genome. The regions corresponding to local peaks of the scores are called as candidate breakpoint ranges and bridged to form full structural variants. Each breakpoint range is then scanned for coverage of normal mate pairs to identify a sub-range with the lowest normal mate pair coverage as the most probable breakpoint locations, and to differentiate homozygous structural variants from heterozygous ones. The supporting evidence of all inversions can be visually inspected using SOLiD™ Alignment Browser (SAB). The output of this pipeline is compared with other in-house and external tools, and the results from PCR validation. For example, we tested 29 out of the 119 inversions found in NA19240, 21 of which were fully validated and 5 were partially validated.

**P11.118****Differential expression profiles in human fetal hemoglobin-expressing and non-expressing tissues**

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The switch from embryonic  $\gamma$ -globin to adult  $\beta$ -globin production is a complex process, involving many factors and regulatory elements, which has not been fully elucidated yet. In an attempt to shed light on the events and genes involved in this maturation process, we established conditions for the generation of primary erythroid precursor cultures from human fetal liver, umbilical cord blood, and adult peripheral blood. These are tissues and cells which express high (fetal liver, umbilical cord blood) and low levels (adult peripheral blood) of  $\gamma$ -globin, respectively. The progress of the cultures was followed microscopically and full growth curves were generated. After culture growth conditions were established, genome-wide patterns of gene expression were investigated for 14 independently derived erythroid precursor cultures using the Affymetrix U133 gene microarray. Differences were observed for a set of approximately 300 genes. At the extreme opposite ends of the  $\gamma$ -globin expression spectrum, four fetal liver and five adult peripheral blood-derived cultures have virtually mutually exclusive patterns of expression for these genes. Five umbilical cord blood-derived cultures lie in the middle of the two extreme expression patterns. Detailed analysis of the genes that exhibit differential patterns of expression will provide more clues as to the regulation of the human globin switching process.

**P11.119****Whole transcriptome analysis of total human RNAs by massively parallel sequencing on the SOLiD™ system**

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Detailed analysis of the entire transcriptome of higher organisms is for the first time demonstrating the complexity of the structure of RNA and providing a better understanding of the role different types of RNA play in the control of gene expression. We report here the results obtained using a prototype version of a human whole transcriptome system being developed by our company. Using total RNA from HeLa and Human Brain, we show the performance of this integrated system in terms of maintaining sample representation, strandedness, and reproducibility. A synthetic RNA spike-in mixture, containing six heterologous transcripts were added to total RNA at different concentrations. Sequencing shows the expected dose dependent response curve and uniform coverage across each transcript. We highlight reproducibility of the system from data generated from six independent field confirmation sites. The results show the protocol is robust and easy to use. The sequencing data across all 6 sites shows similar detection of unique map-

pable sequences, presence of Ref Seq, and coverage across genes. Libraries representing the transcriptome were constructed from as little as 0.4 ug of rRNA-depleted RNA without appreciable loss of coverage. The correlation among the sites for Ref Seq was  $> 0.90$ . We cloned and sequenced rRNA depleted total RNA. Additionally, the same RNA samples were cloned and sequenced using the same protocol by 6 independent field confirmation sites. In addition to technical reproducibility, we will show the ability of the system to detect rare transcripts, alternative splicing events as well as putative fusion transcripts.

#### P11.120

#### Expression analysis of immunorelevant genes in type 1 diabetes pathogenesis

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We analysed expression of genes relevant to immunoregulation in peripheral blood mononuclear cells (PBMC). We compared basal expression versus expression after stimulation with diabetes-associated beta-cell autoantigens. For some parameters (some Th1, Th2, Th3 and Th17 cytokines) we correlated gene data with protein microarray results.

Gene expression of 58 genes of immune regulation was analysed using high density Phalanx gene microarray, containing total 30968 genomic probes. Cytokines were detected by ELISA and quantitative protein microarray. PBMCs were stimulated by diabetogenic peptides (3 GAD65 derived peptides, IA2-peptide and proinsulin peptide). Study cohort consisted of 6 patients with T1D, 14 of their first-degree relatives (5/14 positive for at least one autoantibody - DRLpos group) and 4 healthy controls.

The highest gene expression after specific stimulation was observed mainly within the DRLpos group. These persons are autoantibody positive but with normal intravenous glucose tolerance test. After specific stimulation in DRLpos group, significant gene expression was observed for: IFN-gamma, IL-1,-2,-6,-13,-22,-31, GATA-3, JUNB, IL-6R, STAT-6, TGF-beta. The most important difference was observed for IL-23R which was downregulated in DRLpos group (12 fold) and also in T1D patients (23 fold). In T1D patients we observed an important activation of IL-2,- IL-33, JUNB genes after specific stimulation and strong downregulation of IL-4 and slightly downregulation of STAT-6 and GATA-3. Protein array data are in agreement with gene expression analysis results.

Our results indicated that Th2/Th17 imbalance along with Th1 predominance may be important in human T1D pathogenesis but further study is necessary.

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#### P11.121

#### Ultraconserved elements are enriched among pathogenic copy number variants causing mental delay and congenital anomalies

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The ultraconserved elements (UCEs) are defined as stretches of at least 200 base pairs of DNA that match identically with corresponding regions in the mouse and rat genomes, albeit their real significance remains an intriguing issue. These elements are most often located either overlapping exons in genes involved in RNA processing or in introns or nearby genes involved in the regulation of transcription and development. Interestingly, human UCEs have been reported to be strongly depleted among segmental duplications and benign copy number variants. No comprehensive survey of a putative enrichment of these elements among pathogenic dose variants has yet been reported.

A survey for UCEs was performed among the cryptic genomic rearrangements detected in our series of patients with idiopathic neurodevelopmental disorders associated to congenital anomalies. A total of 25 different elements, out the 481 described UCEs, were contained in 10 of the 21 pathogenic gains or losses detected in our series, what represents a highly significant enrichment of ultraconserved elements.

We therefore propose that these elements may be interpreted as hallmarks for dose-sensitive genes, particularly for those genes whose gain or loss may be directly implied in neurodevelopmental disorders.

#### P11.122

#### Effect of Semax and PGP treatment on expression of Vegf in ischemic rat brain

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Vascular endothelial growth factor (Vegf) is a hypoxia-inducible angiogenic peptide with recently identified neurotrophic effects. On the other hand early post-ischemic delivery of Vegf increased blood-brain barrier leakage and tissue damage. We analyzed the effect of synthetic polypeptide Semax (Met-Glu-His-Phe-Pro-Gly-Pro) and its C-terminal fragment Pro-Gly-Pro upon expression of Vegf in rat brain after global cerebral ischemia. The study was carried out on 2-3-month-old male Wistar rats (n=85). After 15 minutes of irreversible bilateral common carotid artery occlusion the animals were exposed to intraperitoneal injection of either Semax, PGP or saline 1, 4 and 8 hours after the occlusion. Ischemic rats injected with saline were used as control groups. The mRNA expression of Vegf was assessed by relative quantification using real-time RT-PCR. Gapdh was used as the reference gene. The level of Vegf mRNA was decreased compare with control animals: in cortex of sham-operated and rats treated with Semax at 4h, 8h and 24h after occlusion; in hippocampus of rats treated with PGP - at 12h, 24h and treated with Semax - at 4h. The level of Vegf mRNA was increased: in cerebellum of rats treated with PGP - at 12h, and in hippocampus of rats treated with Semax - at 24h.

#### P11.123

#### GPGraphics: A Universal Graphical Backend for SNP Microarray Analysis

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Whole genome association (WGA) studies using microarray platforms are currently one of the most popular methods to search for disease-associated genes throughout the human genome. Several analysis programs are available, most of them, however, lack both a graphical user interface as well as graphical output. Most analysis software packages are designed to run in high power computing (HPC) environments, where GNU/Linux and a variety of Unix flavors are the predominant operating systems. These systems are by their very nature rather text- than graphics-oriented, thus making it difficult for a researcher to get an actual overview of the huge amount of data these programs produce. We now present GPGraphics, which can graphically evaluate data from pooling as well as single sample based WGA software, such as GenePool or PLINK. GPGraphics provides a series of mathematical filters to visualize even faint signals in noisy data. Due to the modular nature of the software, the more processing intensive analyses may still be performed on a non-graphical HPC system, while the evaluation of the data generated by those systems can be performed in the graphical environment of a Microsoft Windows computer. Since the software is written for the Microsoft .NET framework, it will even run on non-Windows systems, provided they have a .NET runtime environment fully compatible to the .NET 2.0 specification. The presented software has been successfully used at our institute to analyze whole genome microarray data for both qualitative and quantitative traits, such as pseudoexfoliation syndrome and cornea thickness, respectively.

#### P11.124

#### Transcriptome profiling of Williams-Beuren syndrome

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Williams-Beuren syndrome (WBS), a neurodevelopmental disorder characterized by mental retardation with unique cognitive and personality profile, is caused by an interstitial deletion on chromosome 7q11.23 encompassing 28 genes. Although the primary cause of WBS is well-understood, the molecular basis of the phenotype is largely un-

known. While hemizygosity of the elastin gene has been associated with supravalvular aortic stenosis, studies of patients with atypical deletions and mouse models have suggested that LIMK1, CYLN2 and GTF2IRD1 might play a role in some aspects of the phenotype.

To identify pathways and processes perturbed in WBS, we used microarrays to profile the transcriptomes of skin fibroblast cell lines from eight young WBS and nine age-matched control girls. Using an iterative signature algorithm on our dataset combined with other skin fibroblast transcriptomes publicly available, we identified modules of coherently regulated transcripts. Among our findings, two GABA-receptor genes (GABBR1 and GABRE), one glutamate receptor subunit (GRIA3) as well as a few other genes possibly related to neurological processes (SLT3, GOLSYN and NAV1) were downregulated in WBS patients. Other modules were significantly enriched in cell adhesion and proliferation factors.

These dysregulations, combined with that of at least 10 extracellular matrix proteins, constitute a potential cause for cognitive deficits and other neurological features, as they may influence CNS development by affecting cell and axonal migration. They could also modulate the function of neuronal connections by accelerating or impairing neurotransmitter release and recycling at the synaptic level.

#### P11.125

#### A comprehensive characterisation of human CD46, CD55 and CD59 transgenic swine fibroblasts - a potential source of nuclei for somatic cloning.

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Transgenic swines, especially those expressing, not a single one, but a combination of the complement system regulators are essential to help overcoming a hyperacute rejection (HAR) and necessary to estimate a potential ratio of a strategy collapse.

Single and triple transgenic swine foetal fibroblasts for human coding sequences of CD46, CD55 and CD59 using a promoter of a human elongation factor 1 alpha gene were generated by lipofection method with 80% capacity. After blasticidin selection stable lines were molecularly characterised and checked for transgene integration by PCR. Forward primers were located in the EF-1 $\alpha$  promoter region and reverse primers in the region coding CD46, CD55 or CD59 respectively. Lines with confirmed transgene integration were subjected for characterisation of expression by RT-PCR. The transgene expression and its impact on human complement system was assessed by human complement-mediated cytolysis assay. Human serum (HS) contains complement system components which are the main reason of HAR. Each transgene expressed in single transgenic line had a protective effect on the tested cells in HS cytotoxicity assay. Also in triple transgenic lines the expression of the transgenes had a wide positive impact on the protection of cells from human complement-mediated lysis, however it was not additive. Cytogenetic analysis was performed to evaluate the chromosomal stability in the transgenic cells. Several cytogenetic staining procedures revealed, inter alia, anomalous number of the chromosomes, structural aberration and dicentromeric chromosomes. Only fully characterised cell lines can be used as nuclei donors for a somatic cloning and producing healthy transgenic animals.

#### P11.126

#### SOLiD™ Sequencing of Whole Genome Bisulfite Converted Libraries Prepared from Nanogram Quantities

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DNA methylation is the most characterized epigenetic mechanism, playing an essential role in normal mammalian development and is associated with gene expression and carcinogenesis. In animals, DNA methylation normally involves the modification of cytosine residues at the 5-carbon position. One popular method to study DNA methylation is to treat the sample with bisulfite. Bisulfite converts cytosine to uracil residues, but methylated cytosines are not affected. Hence, the DNA is subjected to a very specific modifi-

cation dependent on methylation and sequencing of bisulfite converted DNA can yield detailed information about modified segments within a genome. Here we present a novel bisulfite conversion technique that requires only nanogram quantities of genomic DNA. The methodology was applied to two different genomes: Dh10b and Yoruba. Fragment libraries were constructed with methyl C protected adaptors and libraries were sized on Polyacrylamide gel. The DNA was bisulfite converted while still embedded in the gel piece and the library was amplified using an in-gel PCR method. These libraries were then sequenced on the SOLiD™ System, generating 50 bp reads. The high throughput of the SOLiD™ System and the unique advantages of two base encoding allow the researcher to study the methylation of an entire complex genome in unprecedented detail. The results from SOLiD™ sequencing of the converted genomes and the analysis will also be discussed.

## P12. Molecular basis of Mendelian disorders

#### P12.001

#### The novel IVSII-I (G>A) splice donor site mutation on the alpha 1 globin gene

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More than 30 different point mutations and small deletions and insertions have been reported in alpha globin gene. Point mutations are less common, but they may occur at high frequencies in certain areas under selective pressure by malaria. Here we report the combination of IVSII-I (G>A) mutation at  $\alpha 1$ -globin gene and  $\beta$ -HbD(CD121).

After obtaining informed consent, blood samples (10mL) were collected in tubes containing EDTA and extracted by salting out method. Multiplex Gap PCR and direct  $\alpha$ -globin gene sequencing techniques were used to analyze alpha globin gene mutations. Exon 3 of  $\beta$ -globin gene was amplified for determination of HbD variant and digested by EcoRI.

Here we report a novel mutations causing  $\alpha$ -thalassemia that has not been reported previously. The IVSII-I(G>A) mutation in  $\alpha 1$ -globin gene detected in the family with thalassemia phenotype. This mutation disrupts donor splice site of second intron of  $\alpha 1$ -globin gene. Direct sequencing revealed that the proband (7 years old child) was homozygous and the parents were heterozygous carrier. The other nucleotide change for this proband and her mother, was  $\beta$ :CD121 GAA>GCC (known as HbD Punjab). This mutation has not been reported in globin gene server. Because it disrupts the splice site of second exon, so it could be a pathologic mutation. It seems that the homozygous form of this mutation does not make HbH disease (in our case Heinz bodies were negative) and heterozygous from make a mild  $\alpha$ -thalassemia trait.

#### P12.002

#### Molecular analysis of $\gamma$ -globin promoters, HS-111 and 3'HS1 in beta thalassemia intermedia patients associated with high levels of HbF

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We have studied the nucleotide variations in promoter region of gamma globin genes, HS-111 and 3'HS1 regions in  $\beta$ -thalassaemia intermedia, thalassemia major patients and normal individuals from Iranian origin. The five nucleotide variations in the 5' sequences of the Ay globin gene including -369 (C > G), -611 deletion T, -603 GA>AG in all samples, -588(A>G) in heterozygous and homozygous form and -AAGC at -222 to -225 were found with different frequencies. In our study the -369(C>G), -611(-T), -603/604(GA>AG) mutations might as common polymorphism in our population, didn't show any effect on expression of HbF and not correlated with  $\beta$ -globin mutations, Whereas most of the -588 (A) variation is related to  $\beta$ -thalassemia intermedia patients especially in IVSII-I/IVSII-

I status. We also show that the HS-111 (-21 A>G) variation correlates with increased fetal hemoglobin production in β-thalassemia intermedia and major patients. In contrast, the 3'HS-1 (+179 C>T) mutation is not statistically significant. We conclude that the -588 A>G and HS-111 (-21 A>G) variations are useful genetic determinants for differentiation of β-thalassemia major and intermedia patients. However this nucleotide change alone may not be sufficient to raise the level of HbF, other unknown factors may play a role in HbF production.

#### P12.003

#### Further analysis with Multiplex Ligation dependent Probe Amplification (MLPA) of the ABCA4 gene in Spanish patients with retinal dystrophies

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**Introduction:** ABCA4 mutations have been associated with autosomal recessive Stargardt disease (arSTGD). A few cases with autosomal recessive cone-rod dystrophy (arCRD) and autosomal recessive retinitis pigmentosa (arRP) have also been found to have ABCA4 mutations. Comparative genetic analyses of ABCA4 variation and diagnostics have been complicated by substantial allelic heterogeneity. The objective of this study was to determine whether deletions and duplications in the ABCA4 gene are a frequent cause of retinal dystrophies among Spanish patients.

**Subjects And Methods:** We analyzed a total of 55 unrelated families. Mutation analysis was performed in 40 arSTGD families, 6 arCRD families and 9 arRP families. DNA samples from patients were previously studied with the ABCR400 genotyping microarray. Patients with either none or only one mutant allele were analysed with multiplex ligation dependent probe amplification (MLPA). Sequencing was employed for the study of 50 control samples.

**Results:** MLPA allowed us to find one novel mutation in heterozygosis (p.Gln841Lys) in exon 16 of the ABCA4 gene. This variant was located in the target site of the probe. Thus, a lower peak was shown in the pattern of peaks of the MLPA. The p.Gln841Lys mutation was not found in 100 control chromosomes. Neither deletions nor duplications were found.

**Conclusions:** MLPA was mainly designed to detect deletions and duplications of one or more exons of the ABCA4 gene. However, these types of mutations are not a frequent cause of these retinopathies. Nevertheless, this technique enabled us to additionally detect point mutations.

#### P12.004

#### Progressive Familial Intrahepatic Cholestasis type 3: ABCB4 mutations in familial cases

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The ABCB4 protein translocates phosphatidylcholine from the inner to the outer leaflet of the canalicular membrane of the hepatocyte ("flopase" activity). Severe ABCB4 deficiency causes Progressive Familial Intrahepatic Cholestasis type 3 (PFIC-3) characterized by early onset of persistent cholestasis that progresses to cirrhosis and, frequently, to end-stage liver disease before adulthood. We enrolled 132 children with PFIC-3 phenotype and 100 healthy subjects and sequenced all the 27 coding exons of ABCB4 gene. We observed 31 distinct disease associated-mutations in 27 patients from 22 families including 4 families with more than 1 affected sibling. In family A, two brothers carried two null alleles that caused death and liver transplantation at the age of 5 and six years, respectively. In family B two siblings, aged 12 and 2 respectively, carried two missense mutations (compound heterozygous) associated to compensated cirrhosis on both, and with portal hypertension in the oldest. In family C two siblings, a 6-year-old girl and a 4-year-old boy, carried three missense mutations with double paternal mutant allele associated with compensated cirrhosis in both

brothers and episodes of clinical cholestasis in the oldest. In family D, three siblings carried three missense mutations with double maternal mutant allele; liver transplantation was required in the 17 year old boy, compensated cirrhosis was documented in the 12 year old boy whereas any relevant clinical symptom was found in the youngest. ABCB4 deficiency associated with two mutated alleles and severe liver failure in families with affected PFIC-3 children requires a careful genetic counselling.

#### P12.005

#### Novel mutations in the ABCR gene associated with Stargardt maculopathy in the Italian patients

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Stargardt disease (STGD) is a progressive juvenile-to-young adult-onset macular degeneration inherited as an autosomal recessive trait (arSTGD); mutations in the ABCR (photoreceptor-specific ATP-binding cassette (ABC) transporter) gene are responsible for arSTGD. In this study we determined the mutation spectrum in the ABCR gene in a group of Italian patients with arSTGD.

93 families from central Italy, some members of which were affected by autosomal recessive Stargardt disease, were examined.

In all these patients we reported some mutations of ABCR gene. 99 mutations were identified: 61 missense mutations (P68L, I73T, N96K, I156V, G172S, H193P, R212C, N415K, L541P, E616K, R653C, G690V, V767D, W821R, M840R, G863A, T897I, V931M, N965S, T970P, T977P, G978D, F1015I, T1019M, A1038V, R1055W, G1078E, E1087K, T1089I, R1098C, R1108C, R1108H, L1201R, D1204N, P1380L, V1433I, L1473M, P1484S, T1526M, L1580S, A1598D, S1696M, Y1754C, A1762D, A1794D, N1805D, S1806N, H1838D, H1838N, R1843W, G1961E, L1970F, G1977S, L2027F, V2050L, E2096K, L2140Q, K2172R, L2221P, R2269Q, Q2272K); 12 nonsense mutations (Q21X, R572X, W700X, E1087X, S1099X, C1177X, Q1332X, W1408X, W1461X, W1479X, R2030X, Q2220X); 12 splicing mutation (V256splice, IVS6-1G>T, IVS9+1G>C, IVS13+15G>A, Q1376splice, IVS28+5G>A, IVS32+1G>A, IVS35+2T>C, IVS40+5G>A, IVS42-2delA, IVS42+4delG, IVS45+1G>C); 8 small deletions (811delGAGA-TG, 4733delCGTT, 5109delG, 5917delG, 5961delGGAC, 6535delT, 6750delA, 6758delA); 5 small insertion (250insCAA, 324-327insT, 3584insGT, 6548insTGAA, 6464ins8bp); and one gross insertion (4021ins24bp). G1961E was the most frequent in our series. 41 mutations had not been previously described and were not detected in 150 unaffected control individuals..

These data confirm the extensive allelic heterogeneity of the ABCR gene, in agreement with previous observations in patients with Stargardt disease from Italy.

#### P12.006

#### Identifying new AIRE interacting protein.

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Autoimmune polyglandular syndrome type 1 (APS1), is a rare, monogenic autoimmune disease caused by mutations in the Autoimmune Regulator (AIRE) gene. The clinical phenotype of APECED patients reveals a triad of main manifestations: adrenocortical failure, hypoparathyroidism and chronic mucocutaneous candidiasis. The AIRE protein contains several functional domains which are suggestive of a role as a transcriptional regulator: four LXXLL motifs, one SAND domain, one HSR domain and two PHD fingers. Herein we describe the functional studies performed to identify new AIRE interacting proteins. We screened a human thymus cDNA library through the yeast two hybrid technique and we identified seven different clones encoding the same protein. The functional domains involved in the interaction with AIRE protein have been mapped using deletion mutants. The physical interaction was further confirmed with several *in vivo* and *in vitro* approaches in fact we proved the interaction in mammalian cells by co-IP and confocal analysis and then through GST-pull down assays. Chasing AIRE interacting proteins allowed us to discovery a new pro-

tein partner which interestingly shows involvement into apoptosis phenomena and transcriptional regulating properties as well. These data provided by our study will provide new and important insights into AIRE molecular action and regulation.

#### P12.007

#### Review of molecular protocol of Alagille syndrome: what's old, what's new, what's overcome, what's useful

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Alagille syndrome (AGS) is an autosomal dominant multisystem disorder involving primarily liver, heart, eyes, face and skeleton. Mutations in JAG1 are associated with the majority of cases of AGS and haploinsufficiency is the pathogenic mechanism most involved. At the beginning, the genetic testing used in AGS was based on point mutation scanning methods and on FISH to identify JAG1 deletion with a detection rate of 70% and 5% respectively. Later, on 2006, mutations in NOTCH2 (<1%) were identified in AGS patients. In the last years, the technological advances, associated to cost and time reduction, led to an improvement of molecular analysis of this inherited syndrome. From 2002 to now on, 38 patients with clinical diagnosis of AGS were referred to our laboratory. We identified 31 mutations (82%) by JAG1 sequencing: 6 missense, 13 frameshift, 9 nonsense and 3 splice site mutations. Subsequently we performed JAG1-MLPA on remaining negative cases. We found a partial deletion (from exon 22 to 26) and a complete deletion in another patient. The remaining five cases were analyzed by NOTCH2 sequencing and no pathogenic mutations were found. The total detection rate for JAG1 analysis was 87%. So, the "new" method of direct sequencing analysis has replaced the "old" mutation scanning protocol and MLPA, leading to detection also of partial deletion of JAG1, had "overcome" FISH. This new comprehensive JAG1 molecular analysis is "useful" to reach a higher mutation detection rate in AGS patients in short time, while NOTCH2 mutations seem to be rarely associated to AGS.

#### P12.008

#### The novel mutation in JAG1 gene leading to early liver failure in patient with Alagille syndrome

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Alagille syndrome (OMIM 118450) is an autosomal dominant disorder associated with abnormalities of the liver, heart, skeleton, eye and kidneys and a characteristic facial appearance. Clinical picture is variable, major contributors to morbidity arise from bile duct paucity or cholestatic liver disease and severe heart disease. The syndrome is caused by dominant mutations in *jagged1* gene (JAG 1), which encodes a ligand in the Notch-signaling pathway.

We present clinical, histological and molecular data of the patient with Alagille syndrome with early development of liver failure and previously not described mutation in JAG1 gene.

The patient was born 3G/3P; his birth anthropometry was according to gestational age. Due to congenital heart defect (coarctation of aorta and peripheral stenosis of pulmonary arteries) and cholestatic liver disease Alagille syndrome was diagnosed at the age of 3 months. Cholestasis was rapidly progressive and the liver failure developed at the age of 5 months. Child died at the age of 7 months due to the liver insufficiency. Histological findings from liver biopsy revealed infantile giant cell hepatitis. Due to unusual clinical course of the disease molecular study of JAG1 gene was performed. The heterozygous insertion c.2275dupT causing premature stop codon was identified in exon 18 of the JAG1 gene. This mutation was not present in both parents. Conclusion: the previously not described mutation leading to the truncated JAG1 protein was most likely the cause of early development of liver failure in the patient with Alagille syndrome.

#### P12.009

#### Alkaptonuria updated, twelve different HGO mutations identified in Slovakia

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Alkaptonuria (AKU) is an autosomal recessive disorder caused by mutations in the homogentisate 1,2 dioxygenase (HGO) gene leading to deficiency of HGO activity. AKU is characterized by homogentisic aciduria, ochronosis and ochronotic arthritis. In contrast to most of other metabolic disorders, intellectual capacity and life expectancy are not reduced in AKU; however, ochronotic arthritis can be painful and disabling. So far 74 different HGO mutations have been reported in about 217 families. AKU shows a very low prevalence (1:100,000-250,000) in most ethnic groups but there are countries, such as Slovakia or Dominican republic, in which the incidence of this disorder raises up to 1:19000. In case of Slovak population this is difficult to explain by a classical founder effect since in this relatively small country we earlier identified ten different HGO mutations, and now add two more. One of the new mutations is a novel missense change E178G (c.700A→G), while the other found in two unrelated families is the most frequent European mutation M368V (c.1269A→G). HGO-haplotype analysis indicates that M368V mutation in one family represents recurrent mutational event in Slovakia, while in the other family is associated with haplotype identical with that found in Spanish patient. Our results further underscore allele heterogeneity of AKU in Slovakia.

We also report HGO mutations and haplotypes that we identified in patients from Korea (Q33R (c.265A→G), G152A (c.622G→C)), Turkey (N219S (c.823A→G)), France (IVS7+2T→C (c.636+2T→C), M368V (c.1269A→G), G152fs (c.621insG), G217fs (c.819delG)) and Canada (A122V (c.532C→T)). Overview of all known AKU mutations will be presented too.

#### P12.010

#### Molecular analysis of alpha globin gene mutations among individuals with hypochromic microcytic anemia in kermanshah

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Alpha thalassemia is one of the most common single gene disorders in the world. Because both hemoglobin A and F have  $\alpha$  chains, genetic disorders of  $\alpha$  chain synthesis result in defective fetal and adult hemoglobin production. The genetic incidence for this disease varies between 1% and 98% throughout the tropics and subtropics. Alpha-thalassemia is usually caused by one or more deletions of the alpha-globin chain loci. There are alpha globin gene cluster deletions that underlie  $\alpha$ 0 and  $\alpha$  thalassemia; we analyzed deletion types of alpha thalassemia in kermanshah, a province located in the west of Iran. One of this deletions that have high frequency in the Asia, is a 3.7kb deletion (- $\alpha$ 3.7). After iron deficiency was excluded, 93 individuals with low MCH, low or normal MCV and normal or slightly reduced HbA2 level, were analyzed with used GAP PCR technique. - $\alpha$ 3.7 mutation was identified in 29 patients (31.2%). Based on this study, we suggested that the - $\alpha$  3.7 deletion is a common cause of hypochromic microcytic anemia with low MCH, low or normal MCV and normal or slightly reduced HbA2 level in Kurdish population in kermanshah.

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#### P12.011

#### Comprehensive molecular testing for X-linked Alport syndrome

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Alport syndrome (AS) is a progressive renal disease with cochlear and ocular involvement that progresses to end stage renal disease (ESRD) in young adults although milder cases have been described. The majority of AS cases is X-linked (XLAS) and caused by mutations in the COL4A5 gene. The COL4A5 contains 51 exons with more than 400 mutations reported throughout the gene. We present here a comprehensive molecular testing for XLAS using several technologies and an algorithm that maintains good sensitivity while limiting cost.

For adult onset XLAS, we developed an assay identifying the three most common adult type XLAS mutations in the US; C1564S, L1649R, and R1677Q.

For molecular diagnostic testing of individuals with unknown mutations we developed a DNA sequencing assay to identify point mutations and small deletions and insertions.

Additionally, a Multiplex-Ligation Probe dependant Amplification assay (MLPA) and Nimblegen's Chromosome X Tiling array were evaluated to detect complete or partial (exonic level) deletions and duplications in COL4A5.

We developed a public online searchable data base that contains 446 entries from the literature. In this repository, 88% of the mutations are point mutations and small deletions or duplications and 12% are large rearrangements (deletions and duplications at the exonic level).

Using different technologies to analyze different types of mutations, our laboratory has validated a comprehensive test for XLAS that includes the development of a large mutation data base.

#### P12.012

#### Screening of COL4A5 in Hellenic families from Greece and Cyprus with X-linked Alport syndrome

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Alport syndrome (AS) is a hereditary disease of basement membranes that manifests clinically as a progressive nephropathy variably associated with sensorineural deafness and ocular abnormalities. The most frequent form of AS is the X-linked one (~85%) due to mutations in COL4A5 gene. To date, more than 400 different mutations have been identified in the COL4A5 gene, with "private" point mutations being the most. We detected and studied for the first time in Greece and Cyprus - clinically and molecularly - 11 families with AS. Patients of these families showed some of the main characteristic features of Alport syndrome, including hematuria, proteinuria, chronic renal failure and hearing problems. In some of these families, characteristic AS biopsy was available. X-linked AS was inferred in 9 of these families and COL4A5 mutation screening was undertaken based on either linkage analysis results or clinical features only. Direct sequencing of the 51 exons of COL4A5 gene was performed by the use of an ABI PRISM 3130 Genetic Analyzer. We identified three novel mutations in three of the families, E228X, P628L, 3075delT and one known mutation in two of the families, G624D. Interestingly, the two Greek families carrying G624D mutation have a common haplotype flanking the COL4A5 gene, suggesting a founder effect. In addition, male patients in these families have a later manifestation of the disease. Our results will highly contribute in pre-symptomatic and prenatal diagnosis in these families and will add to the international effort being made for genotype - phenotype correlation in X-linked AS.

#### P12.013

#### Identification of a ALMS1 mutation in a Spanish patient with Alström Syndrome

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Alström syndrome (AS, MIM #203800) is a rare autosomal recessive disorder caused by mutations in ALMS1 gene (chromosome 2p13). It

is a multiorganic disorder characterized by cone-rod dystrophy, childhood obesity, progressive bilateral sensorineural hearing loss, insulin resistance and type 2 diabetes mellitus. Dilated cardiomyopathy occurs in more than 62% of patients. Pulmonary involvement and hepatic, renal and urological dysfunction are frequently observed.

ALMS1 gene consists of 23 exons and encodes a novel protein whose function still remains unclear. However, the ALMS1 protein is widely expressed, and localises to basal bodies of ciliated cells and centrosomes playing a possible role in intracellular trafficking.

We present the case of a girl from a Spanish family. She was referred with nystagmus, obesity, progressive sensorineural hearing loss and short stature. The patient also presented benign acanthosis nigricans, endocrine hypothyroidism and several ophthalmologic findings. Fundus examination showed numerous pigmentary spicules and a diminished caliber on the retinal vasculature. She also presented strabismus and poor visual acuity. Electroretinogram (ERG) and visual evoked potentials (VEP) showed no response.

The identification of the mutation was performed amplifying the exons 10 and 16 by PCR, which was followed by direct DNA sequencing. The patient showed a homozygous deletion in exon 16, c.10790\_10791delTG that causes a premature termination codon at amino acid 3600 (p.V3597fsX3600) of ALMS1 and truncation of the protein. In conclusion, one mutation in the ALMS1 gene causative for AS has been reported, proving that mutational screening is a useful tool in the molecular diagnostic of AS.

#### P12.014

#### Early-onset Alzheimer's disease due to novel mutation in PSEN2 gene? - case report

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Patients with an inherited form of Alzheimer's disease (AD) carry mutations in the presenilin genes (PSEN1, PSEN2) or the amyloid precursor protein gene (APP). These disease-linked mutations result in increased production of the longer form of amyloid-beta, the main component of amyloid deposits found in brains of AD patients. Presenilins are postulated to regulate APP-processing through their effects on gamma-secretase, the APP-cleaving enzyme.

We present a 67-year-old woman with family history of dementia, who developed AD in her forties.

DNA was isolated from peripheral blood leukocytes. Intrinsic primers were used to amplify and to sequence exons 3-12 of the PSEN1, PSEN2 genes and exons 16, 17 of the APP gene. APOE status was determined. Blood samples of family members were not available for testing, therefore co-segregation of the novel mutation and phenotype in the affected family was not performed. 100 elderly healthy subjects (aged >65) were tested for p.P69A substitution using PCR-ARMS.

Using sequencing analysis we found a novel mutation (p.P69A) in exon 5 of the PSEN2 gene. This mutation is not found in AD&FTD Database, 2009. No p.P69A mutation was found in the healthy subjects.

The absence of the p.P69A mutation in 100 healthy subjects suggests that the mutation is not a „silent“ polymorphism. The pathologic consequences are uncertain and needs further investigation.

In conclusion, we present a 67-year-old woman, who developed AD in her forties. We found a novel mutation (p.P69A) in the PSEN2 gene, these was not found in the control group. The pathologic consequences needs further investigation.

#### P12.015

#### Molecular genetic findings of familial Alzheimer's disease and familial frontotemporal lobar degeneration in a patient cohort in Portugal - description of five novel mutations

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We studied 127 unrelated patients (aged 64±10 years) with Alzheimer's disease (AD) and frontotemporal lobar degeneration (FTLD) for mutations in the amyloid precursor protein gene (APP), presenilin 1 gene (PSEN1), presenilin 2 gene (PSEN2), microtubule associated protein tau gene (MAPT) and progranulin (PGRN) genes. Until now only 44 mutations in MAPT and 64 in PGRN have been described. To

further clarify the proportion of FTLD and AD cases attributable to mutations in these genes, the frequency of the mutations in a Portuguese clinical series of familial FTLD and familial AD patients referred to our laboratory for genetic testing between 2005 and 2009 was evaluated. Fourteen patients were genetically tested only for AD, 60 only for FTLD and 53 for both diseases with PCR and direct sequence analysis. Eleven different mutations have been identified in 14 out of the 127 patients: two known and one novel missense mutation in the MAPT gene and four novel PGRN mutations. Three of these PGRN mutations caused frameshift while one resulted in abnormal splicing as demonstrated with RT-PCR. Functional tests in cell lines are still running. Two known mutations in PSEN1 gene and one known mutation in PSEN2 were also found.

According to the initial clinical diagnosis mutation frequency in MAPT and PGRN were in the lower range of those described in other studies. The five novel mutations found in MAPT and PGRN suggest that both genes should be tested routinely in patients presenting clinically with FTLD.

#### P12.016

##### Molecular spectrum of androgen receptor gene alterations in Belgian patients.

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Alterations throughout the androgen receptor (AR) gene influence male reproductive development and function and are associated with androgen insensitivity syndrome (AIS), a disease with a prominent genotypic-phenotypic heterogeneity.

We determined the AR gene sequence in 21 patients. Ten patients were suspicious of AIS. 4/6 with known 46,XY karyotype had complete AIS (CAIS) and 1/6 had premature amenorrhea. The presence of an Y-chromosome was confirmed in 2 patients by AR-MLPA, whereas karyotypes remained unknown for 2 AIS patients.

Three nonsense mutations were discovered at codons 353, 829 and 831 respectively, and a 4bp duplication c.2227\_2230dupATGG were found to induce a premature stopcodon at codon 769. Two missense mutations at codon 700 and 860 were detected within the AR ligand binding domain. Suspicion of AR-gene or -exon deletion in 2 patients, was corroborated by AR-MLPA, demonstrating the importance of confirmation methodology. For the remaining 6 patients (few clinical and/or karyotype information was available and) no AR-alterations were found. Carrier status was confirmed in 5 investigated relatives of some of the index cases.

Our study has revealed a number of previously undescribed amino acid alterations presumably causing impairment of the AR protein function.

Our findings demonstrate that AR sequencing and MLPA are important tools to support the clinical diagnosis and counseling of AIS. Moreover, the AIS genetic testing may be used for prenatal diagnosis and pre-implantation genetic diagnosis, for which the CAG-repeat in exon 1 can be used as marker.

#### P12.017

##### TRAF6 dependent EDARADD ubiquitination is necessary for NF-κB activation and is impaired in Anhidrotic ectodermal dysplasia

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Anhidrotic ectodermal dysplasia (EDA) is a disorder characterized by sparse hair, abnormal or missing teeth and inability to sweat. EDA has been ascribed to at least three genes encoding ectodysplasin (EDA1), EDA-receptor (EDAR) and EDAR associated death domain (EDAR-ADD). Ectodysplasin bind to its receptor, EDAR which in turn recruit EDARADD to activate NF-κB downstream signalling pathway. EDAR/NF-κB signalling is necessary to skin appendages developmentdevelopment.

Through a yeast two-hybrid screening of keratinocytes cDNA library, using EDARADD as a bait, we have isolated TAB2 as a partner of EDARADD and demonstrated the involvement of TAB2/TRAFF/TAK1 complex in EDAR/NF-κB NF-κB signalling pathway. TRAF6 is as an

E3 ubiquitin ligase which plays a key role in skin appendages formation. Indeed, TRAF6-deficient mice showed a similar phenotype to mouse homologous EDA. We have demonstrated that EDARADD interact with TRAF6 via the EDARADD death domain. In addition, we have demonstrated that EDARADD is ubiquitinated and that this ubiquitination is dependent on TRAF6 activity and involve probably lysine 63 ubiquitin chains. We have identified a novel mutation in EDARADD (c.402-407del ; p.Thr135-Val136 del) responsible for a sever autosomal recessive form of EDA which abolished NF-κB activity. Interestingly this mutation impaired EDARADD ubiquitination but not the interaction with TRAF6. Together, our studies showed that EDARADD ubiquitination is required for the ectodysplasin-induced NF-κB activation.

#### P12.018

##### Combined indirect strategy for efficient genetic diagnosis of autosomal recessive retinitis pigmentosa

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Autosomal Recessive Retinitis Pigmentosa (arRP) is a retinal dystrophy characterised by its high degree of genetic and allelic heterogeneity, which makes its molecular diagnosis difficult and tedious. In an effort to significantly reduce the workload, sixteen arRP genes were studied in 199 Spanish families (83 arRP and 116 sporadic cases or sRP) with STR and SNP genetic markers, and were also screened for mutations with a genotyping microarray. Cosegregation and homozygosity analysis were performed after the genotyping process.

The overall power to rule out genes of both the STR and SNP methods is very similar, with an average of 11.17 genes (69.82%) and 11.54 genes (72.14%) respectively, taking only into account those families with 2 or more affected genotyped individuals. Twice as many homozygosity alerts were obtained with SNPs, but the ones in common with the STR analysis matched the mutated genes in all 4 fully-characterised control families.

Our study allows discarding many genes and prioritising the mutational screening of the remaining ones, based on homozygosity. Both indirect approaches give similar results when discarding genes. A diagnostic method for arRP and sRP families is proposed, which maximises the probability of reaching a successful molecular diagnosis for each family, making a more rational and cost-effective use of resources by means of computer automation and the combined use of STRs and SNPs.

#### P12.019

##### Genetic diagnosis of Ataxia Telangiectasia and role of Mitochondria on it

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Ataxia telangiectasia (AT) is an autosomal recessive disorder. Although the preferred method is the direct mutation analysis of the ATM gene, the large size of the ATM gene with 63 exons and the large number of possible mutations in patients considerably limit efficiency of mutation analysis as a diagnostic choice. Indirect molecular diagnosis using ATM-related molecular markers facilitates prenatal diagnosis of AT children. In 1/2 Q1\_ this study, four molecular markers: D11S2179, D11S1787, D11S535, D11S1343 are genotyped in 19 unrelated families from different regions of Iran. Amplified products by PCR method were separated using denaturing PAGE gels. In all families, segregation of alleles was according to Mendelian inheritance, and affected chromosomes were distinguishable from unaffected ones. All carriers and affected patients were diagnosed accurately. Thus, this method is effectively useful in prenatal diagnosis of AT.

We also investigated mt-DNA deletions and haplogroups in AT patients. In this study, 24 Iranian patients suffering from AT and 100 normal controls were examined. mt-DNA examined by 6 primers for existence of mitochondrial deletions. We also amplified and sequenced the mtDNA HVS-I by standard sequencing techniques. mtDNA deletions were observed in 54.1% (13/24) of patients (8.9 kb deletion in all samples,

5.0 kb in one and 7.5 kb in two patients), representing mtDNA damage which may be due to oxidative stress in mitochondria. Our results showed that there is no association between mtDNA haplogroups and AT. This data may indicate involvement of mitochondrial damage in the pathogenesis of AT.

#### P12.020

##### Mutations of the *EPHA2* receptor tyrosine kinase gene cause autosomal dominant congenital cataract

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Congenital cataracts (CCs) are clinically and genetically heterogeneous. Mutations in the same gene may lead to CCs differing in inheritance, morphology and severity. Loci for autosomal dominant posterior polar CC and total CC have both been mapped to the chromosomal 1p36 region harboring the *EPHA2* receptor tyrosine kinase gene. Here, we report mutations of *EPHA2* in three CC families from different ancestral groups. In a Chinese family with posterior polar CC, we identified a missense mutation, c.2819C>T (p.T940I), replacing a critical amino acid that functions at the receptor oligomerization interface. In a British family with posterior polar CC and an Australian family with total CC, we found a frameshift mutation (c.2915\_2916delTG) and a splicing mutation (c.2826-9G>A), respectively. These two mutations are predicted to produce novel C-terminal polypeptides with 39 identical amino acids. Yeast two-hybrid analysis showed stronger interaction between the total CC-associated mutant *EPHA2* and low molecular weight protein-tyrosine phosphatase, a negative regulator of *EPHA2* signaling. Our results implicate the Eph-ephrin signaling system in development of human cataract and provide a novel insight into the molecular mechanism underlying the pathogenesis of human CCs.

#### P12.021

##### The method of high-resolution melting (HRM) in diagnostics of autosomal dominant polycystic kidney disease (ADPKD)

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ADPKD is the most common hereditary renal disease. The disorder is caused by mutations of PKD1 and PKD2 genes. PKD1 gene is the main locus, which is responsible for 85% of ADPKD cases and severe disease course. Screening of PKD1 gene is complicated by its high variability and presence of genomic duplications. Currently, 818 sequential variants have been published in PKD database (<http://pkdb.mayo.edu>), which includes polymorphisms as well as causal mutations. Finding of the best method for PKD1 mutation analysis and identification of its new variants are the main aims of this work.

78 patients were screened in non-duplicated PKD1 region. Another 12 patients were analysed within the whole gene. To find the most effective screening method, several detection techniques were tested: direct sequencing, heteroduplex analysis, denaturing gradient gel electrophoresis and high-resolution melting.

From all techniques used, HRM is the fastest and most reliable method for detection of PKD1 mutants. So far, 32 probably causal mutations have been revealed: 11 nonsense mutations, 14 missense mutations, 4 frameshift mutations, 2 deletions without frameshift and 1 intronic mutation. 25 mutations have not been reported yet.

Determination of localization and type of mutations in PKD1 gene and their genotype-phenotype correlation will improve DNA diagnostics together with assessment of clinical prognosis. At the same time, the results could help to reveal the mechanism of ADPKD pathogenesis. Within this study, HRM is the most suitable method for screening of PKD1 gene.

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#### P12.022

##### Missense autosomal recessive bestrophinopathy (ARB) mutations in bestrophin-1 cause defects in intracellular trafficking and a decrease in chloride channel activity.

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INTRODUCTION: The *BEST1* gene encodes bestrophin-1, a basolateral membrane protein primarily expressed in the retinal pigment epithelium where it may act as chloride channel. Mutations in *BEST1* cause several retinal disorders including Best disease; autosomal dominant vitreoretinochoroidopathy; and autosomal recessive bestrophinopathy (ARB). ARB is consequent upon biallelic mutations in *BEST1* and thought to represent the null phenotype.

OBJECTIVES: To investigate the trafficking and chloride electrophysiology of mutant ARB bestrophin-1 isoforms.

METHODS: Chloride channel function was measured by whole-cell patch-clamping in transiently transfected HEK293 cells, the standard system for this analysis. Cellular localisation was determined by immunofluorescence in transiently transfected MDCKII cells which had been polarized on membrane supports to provide an epithelial model system.

RESULTS: We have now identified 13 mutations in 10 families with ARB suggesting that this is an under recognised phenotype. ARB-associated mutant bestrophin-1 isoforms had an altered intracellular localisation, showing a perinuclear reticular pattern typical of the endoplasmic reticulum (ER). Whole-cell patch-clamping demonstrated that all 9 ARB mutants investigated had a significantly reduced chloride conductance compared to wildtype channels.

CONCLUSIONS: Mutant ARB isoforms of bestrophin-1 are not correctly trafficked to the cell surface and appear to be retained within the ER, thus providing an explanation for the lack of chloride-specific currents. We hypothesise that ER associated degradation (ERAD) of mutant bestrophin-1 isoforms is the disease mechanism underpinning ARB.

#### P12.023

##### ABO Genotyping by Capillary Electrophoresis

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ABO blood-group identification by genotyping is often used in identification of suspects, victims, or missing persons in criminal investigations, and can also be a valuable tool in medical applications where routine serological typing is not feasible. Identification of specific combinations of SNPs in the ABO locus on chromosome 9 can be used to determine ABO blood type. However, most methods of ABO genotyping are either too time consuming [e.g., restriction fragment length polymorphism (RFLP) analysis] or too complex [e.g. single-stranded conformational polymorphism (SSCP)] for routine laboratory use. Furthermore, sensitivity is an important consideration, with ABO typing in forensic cases often relying on very small quantities of genomic DNA. A simple and rapid procedure for a multiplex single-base primer extension reaction for ABO typing using six SNP sites within the ABO gene has been described in the literature. In this study, we explore several DNA extraction techniques to optimize upstream preparation of DNA before amplification and electrophoresis. We also present a method for capillary electrophoresis of such multiplex primer extension reactions with sensitivity capable of accurate genotyping down to 0.1 ng of genomic DNA and improved consistency across instruments and laboratories, along with a robust and rapid secondary analysis workflow.

#### P12.024

##### Clinical and molecular study of 12 tunisian patients with Blepharophimosis-ptosis-epicanthus inversus syndrome

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Blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) is an autosomal dominant disorder where eyelid malformation associated with (type I) or without (type II) premature ovarian failure (POF). It is ascribed to mutations in the forkhead transcriptional factor2 (FOXL2) gene.

The purpose of this study is to identify mutations in FOXL2 in 2 sporadic BPES type I patients, 1 BPES type I family with 3 patients, and 3 families with a total of 7 patients were the type of BPES could not be determined.

Coding regions and nearby intron sequences of FOXL2 were analyzed by direct sequencing. A 30-bp in frame duplication 909 - 938 dup 30 was found in two families with undetermined BPES type , and a c.655C>T mutation in two sisters with BPES type I. 2 mutations in FOXL2 were identified in 3 families, including c.672\_939 dup ( 2 families) and c.655C>T ( 1 family). No mutations were detected in one family. 4 genomic variation were identified in 2 sporadic cases, including c.655C>T (1case) and c.501C<T,c.536c>G, c.869C>A (1case). c.869C>A is a novel genomic variation that result in missense change of the encoded protein, ie. p.Pro290His. This is the first reported mutations of FOXL2 in Tunisian BPES cases. One of the mutations, in-frame 30-bp duplication (909 - 938 dup 30), is one of the most common mutation hotspots in the coding region of FOXL2. In BPES family without FOXL2 mutation, it cannot be excluded that the disorder is caused by a position effect in the surrounding region of FOXL2 gene.

#### P12.025

#### TFAP2A mutational hotspot in individuals with Branchio-Oculo-Facial syndrome

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Branchio-Oculo-Facial Syndrome (BOF) is an extremely rare autosomal dominant disorder characterized by cervical skin lesions or branchial sinus defects where skin can either be aplastic or overlaying. Craniofacial features include low-set ears with malformed pinnae and auricular pits, pseudocleft of the upper lip, or cleft lip/palate, and upper lip pits. Neurologic phenotype is characterized by developmental delay or mental retardation in up to 40% of the patients. An array analysis revealed a 3.2 Mbp deletion on 6p24.3 in a familial case of BOF syndrome (Milunsky et al., 2008). In that publication, five sporadic cases of BOF syndrome have mutations in the transcription factor AP-2 alpha gene TFAP2A, a gene from this 3.2 Mbp interval. We have analyzed two familial cases of BOF and three sporadic cases. The majority of them have postauricular cervical branchial sinus defects with hemangiomatous, scarred skin. Only two individuals showed premature hair greying. We detected a recurring mutation in exon 4 and new mutations only in exons 4, 5, and 6. The sequence of these exons is highly conserved in the animal kingdom from humans to the honey bee; explaining why these exons are almost free of SNP, whereas the other exons display a random arrangement of SNPs in their coding sequence. Our study represents the second mutation report to date on patients with BOF syndrome. A larger cohort is now required in order to delineate the genotype with its corresponding phenotype and may then improve our understanding on the variable phenotypes encountered in BOF syndrome.

#### P12.026

#### Dissecting the origin of the trypsinogen triplication mutation

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We have recently reported that the duplication or triplication of a ~605-kb segment containing the cationic trypsinogen gene (PRSS1) on chromosome 7 cause chronic pancreatitis, by means of quantitative fluorescent PCR (QFM-PCR) and FISH (1,2). We had failed to clone the breakpoint junctions by a combination of QFM-PCR and long-range PCR, an observation inconsistent with a simple recombination mechanism. Here, we unraveled the complex structure of the triplication by CGH: the triplicated ~605-kb segment is followed by an inverted segment of ~90-kb, the latter being normally located >100 kb 3' to the former. This greatly facilitated the task of characterizing the breakpoint junctions. Examination of the junction sequences enabled us to decipher how the triplication was generated. The duplication of the ~605-kb segment plus the inverted ~90-kb segment was generated first, explicable by the model of microhomology-mediated, break-induced serial replication slippage (3,4). The triplication was then generated through non-allelic homologous recombination between the duplication-carrying chromosome 7 sister chromatids during meiosis. Our finding not only potentiated the increasingly recognized importance of break-induced replication in the generation of copy number variations (4-6) but also provided a fascinating example showing how a duplication-derived low copy repeats predisposed to the generation of a triplication.

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#### P12.027

#### Lack of association of functional polymorphisms in the $\alpha$ -subunit of the human epithelial sodium channel and bronchiectasis

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Bronchiectasis is defined as a permanent dilation of the airways arising from chronic bronchial inflammation/infection. In 50% of cases, no etiology can be identified. The role of the epithelial sodium channel ENaC has been pointed out in the pathophysiology of cystic fibrosis. Recently, it has been shown that the common human ENaC alpha polymorphism haA663T is a functional polymorphism that affects human ENaC surface expression. We extensively analysed ENaC in 55 patients with idiopathic bronchiectasis and without two *CFTR* mutations. Thirty-eight patients presented functional abnormalities suggesting impaired sodium transport (abnormal sweat chloride concentration or nasal difference measurement), and 17 had no such evidence. Sequencing of the exons and flanking introns of the ENaC $\alpha$  gene identified three different intronic sequence variations (IVS7+54C>T; IVS11+32G>A, and IVS11-6C>T) and two different amino-acid changes ((3 p.W493R, and 1 p.V562I) in heterozygous state in four patients (3 with impaired sodium transport (7.8%), and 1 without evidence of sodium transport abnormality (5.9%)). Moreover, we studied the distribution of the haA663T genotypes in each group. We observed no significant association between the haA663T genotypes and bronchiectasis with impaired sodium transport. Moreover, the frequency of the A663 allele (associated with a less channel activity) is similar in patients with bronchiectasis with or without impaired sodium transport (66.25% vs 67.6%), and in patients with or without only one *CFTR* mutation (65.9% vs 68.18%). In conclusion, subtle genetic changes in alpha-ENaC subunits might not be at the origin of bronchiectasis in our population.

#### P12.028

#### Functional analysis of missense mutations identified in the PMM2 gene causing congenital disorder of glycosylation type-1a

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The congenital disorders of glycosylation (CDG) affect the synthesis or processing of N-glycans. CDG1a (MIM#212065) type is the most

common form of the disease, and is caused by a deficiency in the cytosolic protein phosphomannomutase (EC 5.4.2.8, PMM 2). The aim of this work has been the functional analysis of 12 missense changes identified in a cohort of Spanish patients in order to provide clues to understand the phenotype-genotype correlation and to investigate new therapeutic approaches. First we have studied the PMM2 protein stability and gene expression in the fibroblast cell lines. All fibroblasts cell lines presented lower amount of PMM2 mutant protein while no effect on mRNA stability was detected. The prokaryotic *in vitro* studies have demonstrated that all new changes are disease-causing mutations and have revealed the presence of null mutations (R123Q, R141H, F157S, P184T, F207S and D209G), mutations with residual activity ranging between 44-54% (L32R, T118S, T237M and P113L) and mutations with residual activity ranging between 16-21% (D65Y and V44A). Most of the patients are functional hemizygous for a null mutation and a mutation with intermediate residual activity -usually associated with a moderate form of the disease- or with a mutation with higher residual activity usually associated with a milder clinical phenotype. The protein stability assays identified at least six changes (V44A, D65Y, F157S, P184T, F207S and T237M) with decreased amount of immunoreactive PMM2 protein and decreased half life compared to wild type, opening up therapeutic possibilities by pharmacological chaperones in several of our cohort of patients.

#### P12.029

##### HLA polymorphism in celiac disease Romanian children

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**Introduction:** Celiac disease (CD) is an immune-mediated enteropathy caused by a permanent sensitivity to gluten in genetically susceptible individuals (DQ2 or DQ8 HLA haplotype). **Objective:** To correlate the clinical forms of CD with villous injury severity, IgA anti-tissue transglutaminase (tTG) antibodies serum level and DQ2 / DQ8 haplotype. **Material and Methods:** We recruited 2 lots: lot 1 - 24 children diagnosed with CD by mass screening in subjects associating risk factors and lot 2 - 24 healthy controls matched with sex and age. HLA DQ2 and DQ8 alleles were typed by PCR-SSP, IgA tTG assessment was made using ELISA and villous injury was classified using Marsh score. **Results:** From 24 CD patients, 15 associated atypical or silent form of disease and only 9 associated the typical form. Haplotype analysis showed that the main combination observed was DQ2 in cis conformation (DQA1\*0501 and DQB1\*0201 alleles) in 14 patients from lot 1 and in one subject from lot 2. We found a strong correlation between IgA tTG serum level and villous injury degree ( $p=0,03$ ), but there was no statistically significant correlation between the clinical forms of disease, Marsh score and the distribution of the different alleles ( $p = 0,07$ ). **Conclusion:** We confirmed in this study the high frequency of DQ2 haplotype in CD patients. Presence of HLA DQ2 or DQ8 is mandatory but not sufficient for developing gluten enteropathy. HLA polymorphism seems to have no impact on clinical forms of CD.

1

#### P12.030

##### A large family with spinocerebellar ataxia type 6 in Iran: a clinical and genetic study

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The authors describe a large Iranian family with autosomal dominant cerebellar

ataxia, which included 14 patients in four generations. We examined seven patients who had expanded CAG repeats in the CACNA1A gene with repeat instability (24 and 25 repeats). Although all patients showed cerebellar ataxia, each patient exhibited peripheral neuropathy or spasticity indicating intrafamilial phenotypic variability. This is the first report of SCA6 in Iran, and suggests the worldwide distribution of SCA6. **Key words:** Spinocerebellar ataxia , SCA6 , Autosomal Dominant , Iran

#### P12.031

##### Somatic and germline mosaicism in a carrier of a large deletion at Xp21 locus leading to chronic granulomatous disease and McLeod syndrome

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The proband is a boy who presented when he was three months old with a granulomatous lymphadenitis. Nitroblue tetrazolium reduction assay (NBT test) was consistent with the diagnosis of chronic granulomatous disease (CGD), a immunodeficiency disease resulting from mutation in genes encoding the subunits of NADPH oxidase complex : CYBA, CYBB, NCF1 and NCF2 (Online Mendelian Inheritance in man MIM#s 233690, 306400, 233700, 233710). Western blot analysis of the NADPH oxidase subunits revealed absence of expression of GP-91phox encoded by CYBB (Xp21). The patient had also acanthocytosis suggesting a McLeod syndrome that is caused by a mutation in XK locus also at Xp21. We hypothesized a contiguous gene deletion for XK and CYBB. We confirmed the presence of a large deletion of 1.8Mb of CYBB by CGH array and characterized precisely the breakpoints by sequencing a PCR fragment generated using primers from each side of the deletion. Using quantitative PCR at the CYBB locus and the specific PCR amplification of the deleted X chromosome, we showed that the mother had a somatic mosaicism and that an half sister of the proband was a carrier of the deletion. Segregation analysis of microsatellite markers in this family (including 3 additional half brothers of the proband) revealed that the deletion was either present or absent on the same X-chromosomal region inherited from the mother, thus indicating a germline mosaicism. We provide here molecular evidence for the occurrence of somatic and germline mosaicism in a carrier of CGD and McLeod syndrome.

#### P12.032

##### Chiari Malformation Type I: Linkage to chromosome 16p13.3 in a large Spanish kindred

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**Background:** Chiari malformation type I (CMI) is a mesodermal anomaly that may cause severe and progressive neurological deficits. The main feature is the ectopia of the cerebellar tonsils which are downwardly displaced through the foramen magnum. Familial aggregation, twin studies and cosegregation with known genetic syndromes support that at least in a subset of CMI patients there is a substantial genetic contribution, but up to date no genetic loci or genes have been convincingly linked to CMI.

**Objective:** To map the disease-causing gene in a large Spanish kindred with Chiari malformation type I (CMI) with a mendelian pattern of inheritance.

**Methods:** DNA samples from 31 family members were obtained.

Twelve individuals were classified as affected after determine a downward herniation of cerebellar tonsils of  $\geq 3$  mm through the foramen magnum and a volume reduction of the posterior fossa in a MRI sagittal view. After carrying out cytogenetic analysis to rule out major chromosomal rearrangements, a single nucleotide polymorphism (SNP)-based, 0.62cM density genomewide scan was performed in the 21 first recruited individuals.

**Results:** Preliminary linkage analysis considering 21 individuals revealed a disease locus in a 4.3Mb region on 16q13.3 with a maximum multipoint parametric LOD score of 3.109. Several candidate genes map to this region. Further analysis will include ten recently recruited individuals and additional microsatellite markers.

**Conclusions:** A genetic locus in CMI has been described, underscoring the monogenic character of the disorder in some families. Elucidation of the putative disease-causing gene in 16p13 awaits further investigation.

#### P12.033

##### Genetic Heterogeneity of Geleophysic Dysplasia

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Geleophysic dysplasia (OMIM 231050, GD) is an autosomal recessive disorder characterized by short stature, small hands and feet, cone-shaped epiphyses, delayed bone age and shortened tubular bones. Patients present with a progressive cardiac disease with dilation and thickening of the pulmonary, aortic or mitral valves often leading to death before 5 years of age. Studying six GD families, we mapped the disease locus gene on chromosome 9q34.2 and identified four distinct missense mutations and a nonsense mutation in the A Disintegrin And Metalloproteinase with Thrombospondin repeats- like 2 gene (ADAMTSL2). The ADAMTS-like subfamily comprises proteins homologous to the ADAMTS ancillary domains but lacking the protease domain and hence lacking catalytic activity. Their functions are yet unknown. Using a yeast two hybrid screen, we identify Latent TGF $\beta$  Binding Protein 1 (LTBP1) as a partner of ADAMTSL2. We also found a higher level of active TGF $\beta$  and an enhanced level of phosphorylated SMAD2 in GD fibroblasts allowing us to conclude at an enhanced TGF $\beta$  signalling. Following this initial study, we have collected the samples of 18 additional GD families and identified ADAMTSL2 mutations in 6/18 comprising 4 novel mutations. We do not find any distinctive clinical feature between patients with or without ADAMTSL2 mutation. Finally, we also found an increase TGF $\beta$  level in non mutated ADAMTSL2 fibroblasts. We conclude that GD is a clinically homogenous but genetically heterogeneous condition. On going studies will hopefully lead to the identification of another disease gene presumably also involved in the bioavailability of TGF $\beta$ .

#### P12.034

##### DNA-diagnostics of choroideremia in Russian family

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Choroideremia - is a congenital X-linked ocular disease characterized by the degeneration of the choriocapillaris, the retinal pigment epithelium and the photoreceptor of the eye. The disorder leads to the progressive loss of vision beginning at an early age resulting from the complete atrophy of the choroid and retina. The *CHM* gene responsible for choroideremia, is located on Xq21.2, contains 15 exons and encodes a protein, the Rab escort protein-1 (REP1), which is involved in membrane trafficking. Currently, there are about 110 mutations in *CHM* gene, and these are all nonsense, frameshift or splice site mutations leading to choroideremia.

The purpose of our study was elaboration of DNA-diagnostics of choroideremia in affected Russian patient. Sequencing analysis of all exons and intron-exon junctions of *CHM* in affected man showed a previ-

ously described nonsense mutation Arg253Stop (c. 757C>T) in exon 6 of *CHM*. Restriction analysis performed in a sister of the patient, who has small clinical presentations of choroideremia, detected mutation Arg253Stop (c. 757C>T) in heterozygous state. Thus the given DNA-analysis results revealed a disease-causing mutation Arg253Stop (c. 757C>T) and confirm the diagnosis "choroideremia" in the clinical case.

#### P12.035

##### Functional analysis of chronic pancreatitis-associated 5' regulatory variants in the pancreatic secretory trypsin inhibitor (SPINK1) gene

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**Introduction:** The SPINK1 gene, which encodes the pancreatic secretory trypsin inhibitor, is one of the major genes predisposing to chronic pancreatitis. To date, a dozen of variations have been described in the 5' regulatory region (RR) of SPINK1 but their functional effects remain unknown. The aim of this study was to systematically characterize all these currently known 5' RR variants.

**Method:** The wild-type 5' RR of SPINK1 was firstly cloned into the pGL3-Basic Luciferase Reporter Vector. All the 5' RR variations in the SPINK1 gene were then introduced into the pGL3-SPINK1 construct, respectively, by means of site-directed mutagenesis. SPINK1 promoter activities were determined in human pancreatic Colo-357 cells. Functional relevance of some variants was further evaluated by EMSA.

**Results:** The 5' RR variations can be divided into three categories in terms of luciferase expression, which correlated well with clinical findings. The variants that caused a decreased expression often show consistent disease association among different studies whilst those that had no effect on expression often show equal allele distribution between patients and controls. The variants that caused an increased expression are, in fact, in cis with a known disease-causing mutation. EMSA assay demonstrated that variations located in well defined regulatory motifs affected protein-DNA interactions.

**Conclusion:** This work was the first to assess the functional impact of the 5'-RR's SPINK1 variations. Our finding clarified the role of the diverse SPINK1 5' RR variants in the etiology of chronic pancreatitis and resulted in a better understanding of the genotype/phenotype relationship.

#### P12.036

##### Genetic background of primary ciliary dyskinesia in Polish patients - search for mutations in candidate genes

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The study group comprises families with Kartagener syndrome (KS) and CDO (ciliary dysfunction only). **Molecular analysis of the genes involved in PCD pathogenesis.** *DNAH5*. Sixty-eight families were analyzed for the consistency in the inheritance of neutral intragenic SNPs and the disease phenotype. In total, 109 PCD families were screened for the presence of mutations in *DNAH5* exons using SSCP/heteroduplex method. In 77 (of 79) exons analyzed, we identified 13 STOP mutations (including four already reported) and 11 missense not found in the control Polish population. Four mutations (3 STOPs, also found in European patients, and one missense in exon 32 not reported before) were found in more than one family; haplotype background analysis indicated common origin for each of these repetitive mutations. *DNAI1*. The search for mutations in *DNAI1* was conducted in 113 families without *DNAH5* mutations. Among 18 (of 20) exons examined, four harbored mutations (three reported and two newly found). The most frequent were: insertion in intron 1 and missense in exon 17, previously reported among European patients. Mutations in *DNAH5* and *DNAI1* are responsible for PCD/KS in at least 20% and 8% Polish families, respectively, all with ODA defects. **Genetic background of atypically inherited PCD.** Two families with overlapping X-linked RP and PCD symptoms were examined. In one, a mutation in exon 2 of the *XL-RPGR* gene has been identified and shown to cause aberrant

RNA splicing. In the second family, a substitution in intron 2 (+5g>a) has been found with unknown effect on splicing.

#### P12.037

#### Analysis of *RUNX2* in a Danish cohort of cleidocranial dysplasia patients revealed two large chromosomal deletions and 14 pathogenic point mutations

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Cleidocranial dysplasia (CCD) is an autosomal dominant inherited disease caused by mutations in the Runt gene *RUNX2* (alias *CBFA1*; OMIM 600211). No other candidate genes or loci are known from family studies. In a cohort of 19 Danish CCD patients, pathogenic *RUNX2* mutations were found in 16 cases. This represents a higher mutation detection rate than reported by similar studies (1). The mutations represent six missense mutations, two nonsense mutations and four shift mutations plus two large chromosomal deletions. Eight mutations were novel and six were known mutations, and two mutations were found in two families each. The large deletions at 6p12.3-21.1 represent 500 Kbp and 750 Kbp respectively and included exon 1 to 6 of *RUNX2* and the proximal located *SUPT3H* gene. The point mutations were mainly found in the Runt domain and the nuclear leading sequence of *RUNX2*. Two repeat variations were found in the poly alanine and glutamine repeats in three cases. These were judged to be non-pathogenic that additional functional mutations were found in the coding regions. In total 9 different cohorts of CCD patients including this work, represent 199 families and *RUNX2* mutations were found in 124 of these, which represent 61% compared to the 84% in this study. Identification of the two large deletions by mutation screening of CCD patients has not been reported before and suggests that future mutation studies must include analyses for large chromosomal deletions or duplications.

#### P12.038

#### A novel *VPS13B* mutation in two brothers with Cohen syndrome, cutis verticis gyrata and sensorineural deafness

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We have previously described a syndrome characterized by microcephaly, cutis verticis gyrata (CVG), retinitis pigmentosa, cataracts, hearing loss and mental retardation (MIM #605685) in a non-consanguineous Lebanese family. In view of the rarity of the disorder and the high rate of inbreeding in Lebanese, we assumed an autosomal recessive trait inherited from a common ancestor. Indeed, genome-wide linkage analysis resulted in a single region on chromosome 8q22 with homozygosity by descent in the patients, comprising the Cohen syndrome (CS) gene, *VPS13B*. We identified a novel homozygous splice site mutation that activates a cryptic acceptor site in exon 52. CVG and deafness have never been reported in CS. This may reflect a variant of CS. Alternatively, there may be an overlap of genetic conditions: Offspring from consanguineous parents may be homozygous for mutations in unlinked genes. Deafness and CVG could be caused by mutations in different loci. However, our linkage data do not suggest another causative locus. Another mutated gene or modifier locus may segregate *in cis* and be responsible for deafness and CVG, but no deafness locus maps to our 8q22 region. In contrast, the frequent association of CVG with mental retardation suggests that it may be a rare manifestation of CS. The mapping approach conducted here can serve as a paradigm in rare recessive phenotypes: The prevalence of homozygosity for the causative mutations can also be high in families *without* documented consanguinity, due to a distant common ancestor, especially in small populations with a high rate of inbreeding.

#### P12.039

#### Large deletion comprising *COL3A1* causes aortic dissection

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Aortic dissection (AD) is a life-threatening condition associated with high rates of morbidity and mortality. AD can occur non-syndromic, e.g. in the case of familial thoracic aortic aneurysms leading to type A dissections (TAAD), or in association with genetic syndromes, such as Marfan syndrome (MFS) caused by *FBN1* mutations, Loeys-Dietz syndrome caused by *TGFBR1* or *TGFBR2* mutations, and vascular Ehlers-Danlos syndrome (EDS IV) caused by *COL3A1* mutations. Although mutations in *FBN1*, *TGFBR1*, and *TGFBR2* account for the majority of AD cases referred to us for molecular genetic testing, we have encountered negative genetic testing results in a large group of patients, suggesting the involvement of other genes, e.g. *COL3A1*, *ACTA2* or *MYH11*, as the genetic cause of AD. In this study, we have assessed the impact of *COL3A1* mutations in patients with suspected MFS in whom mutation screening in *FBN1* and/or *TGFBR1* and *TGFBR2* revealed no disease-causing sequence variation. MLPA analysis of 133 unrelated patients identified the heterozygous deletion of the entire *COL3A1* gene in one patient with abdominal AD. Subsequent microarray analyses and sequencing of breakpoints revealed the deletion size of 3,408,306bp. Furthermore, DNA sequencing of 29 unrelated patients identified two novel exonic *COL3A1* sequence variants (c.1105G>A and c.1854A>T). Our data not only emphasize the importance of screening for *COL3A1* mutations in comprehensive genetic testing of AD patients with suspected MFS not fulfilling the Ghent criteria, but also extend the molecular etiology of EDS IV by providing hitherto unreported evidence for true haploinsufficiency of *COL3A1*.

#### P12.040

#### Variant phenotype in individuals with severe CYP 21 mutations

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Congenital adrenal hyperplasia(CAH) is a common autosomal recessive disease most frequently occurring due to mutations in the CYP 21 gene. Several "severe" mutations are reported to cause severe salt wasting(SW) form of the disease. However, homozygous carriers of the mutations without clinical symptoms have been described.

Aim. To analyze severe CYP 21 mutations in patients with CAH and their relatives and to analyze genotype/phenotype correlation.

Material and methods. Sixteen children (6 boys and 10 girls) with CAH from 12 families were diagnosed with salt wasting CAH. The diagnosis was confirmed by high 17-OHPProgesterone levels.

Molecular analysis was performed in all patients and 22 first degree relatives with ACRS-PCR method detecting 11 frequent mutations. Total of 66 chromosomes were analyzed, 32 in patients, and 34 in relatives.

Results. Intron 2 splicing mutation (nucleotide 656) was the most common (33/66=50%), followed by codon 318(exon 8) mutation (15/66=22.9%). Complex mutations were present in two children and two relatives. All 16 children with SW had severe mutations. However, 6 relatives were also homozygous for severe or had complex mutations without symptoms. Prenatal diagnosis helped prompt treatment of two newborn boys. Four of the relatives had I2 homozygous splicing mutation. "Leaky" mutation might be an explanation in these cases. However, the complex mutations are more difficult to explain.

Conclusion. Although severe mutations were associated with the SW form, it is difficult to explain the homozygosity in healthy relatives. This complicates the prenatal diagnosis and counseling. Further analysis in individuals with no genotype/phenotype correlation is warranted.

**P12.041****The study of the CYP21A2 gene mutations in children with congenital adrenal hyperplasia from Republic Bashkortostan (Russia)**V. L. Akhmetova<sup>1</sup>, Z. F. Ramova<sup>2</sup>, O. A. Malievsky<sup>2</sup>, E. K. Khusnutdinova<sup>1</sup>;<sup>1</sup>Institute of Biochemistry and Genetics, Ufa, Russian Federation, <sup>2</sup>Bashkir State Medical University, Ufa, Russian Federation.

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders of adrenal steroidogenesis in which 21-hydroxylase deficiency accounts for over 95% of cases.

We studied 68 patients with CAH from 68 families. The patients were divided into 2 groups according to clinical findings: salt wasting (SW) (N=41) and simple virilizing (SV) (N=27). We screened CAH-patients for 11 the most common mutations in the CYP21A2 gene: large gene deletion or large gene conversion (*delA2/LGC*), *G110del8nt*, *P30L*, *I2splice*, *I172N*, *V281L*, *Q318X*, *R356W*, *E6cluster*, *F306+1nt*.

Mutations of the CYP21A2 gene were revealed in 84.51% of the studied CAH-chromosomes. The mutations were distributed as follows: *delA2/LGC* (33.1%), *R356W* (19.72%), *I2splice* (13.38%), *I172N* (8.45%), *Q318X* (7.04%), *P30L* (1.41%) and *V281L* (1.41%). In 15.49% CAH-chromosomes mutations were not identified. We found 8 patients who carried 3 mutations, two from which formed a cluster: *Q318X+R356W* (N=5), *I172N+Q318X* (N=2), *I172N+R356W* (N=1). This complex of alleles probably resulted from large conversions or multiple mutations events.

In patients with SW form the most frequent mutation was *delA2/LGC* (39.08%), while *R356W*, *I2splice* and *Q318X* were found with lower frequencies (25.29%, 16.09% and 10.35%, respectively). In patients with SV form the most common mutations were *delA2/LGC* (25%) identified only in compound heterozygous state and *I172N* (17.86%), followed by *R356W* (10.71%) and *I2splice* (8.93%). Thus, we have been able to detect the spectrum of diagnostic significant CYP21A2 gene mutations typical for SW and for SV forms of CAH patients.

**P12.042****Mutation screening of CYP1B1 Gene in North Indian Congenital Glaucoma Patients**M. Tanwar<sup>1</sup>, T. Dada<sup>2</sup>, R. Sihota<sup>2</sup>, V. Gupta<sup>2</sup>, R. Dada<sup>1</sup>;<sup>1</sup>Dept. of Anatomy, AIIMS, New Delhi, India, <sup>2</sup>Dr. R.P.Centre for Ophthalmic Sciences, AIIMS, New Delhi, India.

Primary congenital glaucoma is an inherited ocular congenital disorder that can result in permanent blindness. Its prevalence varies across ethnic communities, ranging from 1 in 10,000-20,000 in the western populations to 1 in 3300 in Southern India. Aim of this study was to investigate the predominant mutations in CYP1B1 gene in north-Indian PCG patients. Fifty PCG patients and 50 ethically matched controls without any ocular disease were enrolled in the study. CYP1B1 gene was screened for six most prevalent mutations (Termination at 223, Gly61Glu, Pro193Leu, Glu229Lys, Arg368His and Arg390Cys) by PCR-RFLP method. On PCR-RFLP analysis total 20/50(40%) showed either of these mutation. Ter@223 was found in 18%, R390C in 16% and R368H in 8% patients. On DNA sequencing R390C mutation was found to be R390H and three novel (L24R, F190L and G329D) mutations were identified. Because of Ter@223 mutation a functional null protein is produced. Arginine residues at 368 and 390 are highly conserved in cytochrome 450 proteins. These map to helix K, which is involved in proper protein folding and heme binding. E229 amino acid (aa) residue are also conserved in different cytochrome P450 proteins. E229K mutation can cause conformational changes in the protein. The most prevalent CYP1B1 mutation in our population is Ter@223 (18%) followed by R390H (16%) while from other studies from south India R368H was the most prevalent CYP1B1 mutation. Our data is different from southern population it may be because of genetic heterogeneity of the disease and different evolutionary history of both populations.

**P12.043****Hypoplastic left heart syndrome: is it all in HAND?**D. Barachetti<sup>1</sup>, D. Marchetti<sup>1</sup>, F. Seddio<sup>2</sup>, L. Boni<sup>3</sup>, A. R. Lincecco<sup>1</sup>, S. Villagra<sup>3</sup>, A. Mendoza<sup>3</sup>, L. Pezzoli<sup>1</sup>, L. Galletti<sup>2</sup>, P. Ferrazzi<sup>2</sup>, M. Iascone<sup>1</sup>;<sup>1</sup>Genetica Molecolare - USSD Lab. Genetica Medica, Ospedali Riuniti, Bergamo, Italy, <sup>2</sup>Dipartimento Cardiovascolare, Ospedali Riuniti, Bergamo, Italy,<sup>3</sup>Instituto Pediatrico del Corazon Hospital "12 de Octubre", Madrid, Spain.

Hypoplastic left heart syndrome (HLHS) consists of a heterogeneous group of cardiac malformations with various degrees of underdevelopment of the left heart-aorta complex. It is one of the most severe congenital heart diseases and it's usually lethal during early infancy. Most hypotheses formulated to explain the pathogenesis of HLHS assume that there is a primary anatomic/genetic abnormality which results in low flow through the left heart and it is the diminished flow that ultimately leads to growth failure of the left sided structures. The molecular causes of HLHS are unclear. A recent report of mutations in basic helix-loop-helix (bHLH) transcription factor HAND1 found in hearts with HLHS has suggested that in hypoplastic human hearts HAND1 function is impaired. Because of the profound implication of this finding, we attempted to replicate it using peripheral blood samples from 46 patients and 8 different cardiac samples obtained from 5 patients with HLHS who underwent cardiac surgery.

Newborns and children (25% female) with HLHS undergo a complete evaluation to determine the type of defect and the potential presence of extracardiac defect. The mean age at surgical intervention was 8 days old. We sequenced the entire HAND1 gene starting from genomic DNA extracted from peripheral blood lymphocytes and from cardiac samples. No evidence of germline or somatic mutations was found in this study.

Germline and somatic mutations in HAND1 do not seem to be a frequent cause of abnormal cardiac development at basis of HLHS.

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**P12.044****A Chinese patient with congenital nephrotic syndrome of the Finnish type caused by compound heterozygous mutations in NPHS1**Y. P. Yuen<sup>1</sup>, W. K. Siu<sup>2</sup>, S. C. Lau<sup>3</sup>, A. Y. Chan<sup>4</sup>, C. W. Lam<sup>5</sup>;<sup>1</sup>Department of Pathology, Hong Kong, China, <sup>2</sup>Department of Obstetrics and Gynecology, Princess Margaret Hospital, Hong Kong, China, <sup>3</sup>Department of Paediatrics & Adolescent Medicine, Princess Margaret Hospital, Hong Kong, China, <sup>4</sup>Department of Pathology, Princess Margaret Hospital, Hong Kong, China, <sup>5</sup>Department of Pathology, The University of Hong Kong, Hong Kong, China.

Mutations in NPHS1 (OMIM no.256300), NPHS2 (OMIM no.600995), WT1 (OMIM no.194080 and 136680) and LAMB2 (OMIM no.609049) are known to account for the majority of early-onset hereditary nephrotic syndromes. Our patient was the first child of non-consanguineous Chinese parents. She was born prematurely at 34 weeks with a birth weight of 1.98 kg. The placenta weighed more than 53% of the birth weight. She was noted to have facial puffiness, abdominal distension and pitting edema in her extremities on Day 13 after birth. Nephrotic syndrome was confirmed by laboratory investigations. The karyotype was 46,XX and no ocular abnormalities were noted. Left nephrectomy was performed when the patient was 4 months old for deteriorating symptoms in spite of daily albumin infusion and the use of indomethacin and ACEI. Ultrastructural examination of the removed kidney showed narrow slits and absence of slit diaphragm. The entire coding sequences and flanking introns in NPHS1, NPHS2 and WT1 were analyzed by direct sequencing. No pathologic mutations were identified in NPHS2 and WT1. However, two mutations, c.2172\_2173delTG and c.2783C>A, were detected in the NPHS1 gene. The 2-nucleotide deletion is predicted to create premature stop codon resulting in a truncated protein of 737 amino acids (E725GfsX13). The latter mutation changes codon 928 from serine (TCG) to a stop codon (TAG) (p.S928X). Both were novel mutations not described before. This is the first case of genetically-confirmed congenital nephrotic syndrome of the Finnish type in Hong Kong Chinese.

**P12.045****A novel mutation of the ELA2 gene in a patient with severe congenital neutropenia**M. Maschan<sup>1</sup>, M. Kurnikova<sup>2</sup>, A. Maschan<sup>1</sup>, I. Shagina<sup>2</sup>, D. Shagin<sup>2,3</sup>;<sup>1</sup>Federal Research Clinical Center for pediatric hematology, oncology and immunology, Moscow, Russian Federation, <sup>2</sup>Evrogen Joint Stock Company, Moscow, Russian Federation, <sup>3</sup>Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, RAS, Moscow, Russian Federation.

Severe congenital neutropenia (SCN) is an inborn disorder of granulopoiesis. Mutations of the ELA2 gene encoding neutrophil elastase

are responsible for most cases of SCN and cyclic neutropenia (CN), a related but milder disorder of granulopoiesis. We found a novel mutation in *ELA2* in Russian patient with severe congenital neutropenia. The proband was a 2 year old boy, the manifestation of disease occurred at the age of one month with the first of multiple recurrent episodes of tonsillitis, bacterial infections of skin and stomatitis. Absolute neutrophil count below 0,5\*10<sup>9</sup>/l was recorded on multiple occasions. Serum immunoglobuline level, hemoglobin and platelet levels were normal. Bone marrow aspiration showed early arrest of granulocyte maturation. Family history was unremarkable. The clinical diagnosis of sporadic case of severe congenital neutropenia was thus established. Genomic DNA amplification of the coding exons of the *ELA2* gene and their flanking regions were amplified from genomic DNA by PCR and sequenced. The mutation in heterozygous state was revealed in exon 4, and PCR fragment of exon 4 was additionally cloned and sequenced. The novel mutation is a 1-bp deletion in exon 4 (c.545delG) causing frame shift and introduction of a premature stop codon (8 codons downstream of the mutation). This frame shift mutation remove the C-terminal portion of the molecule probably resulting in destabilization of the protein structure near the active site.

#### P12.046

#### Connxin 26 gene in mutations autosomal recessive non-syndromic hearing losses

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Up to day, about 34 genes and more than 100 loci have been detected in the etiology of non-syndromic hearing loss. In the result of mutation scannings carried out in various communities, it has been indicated that *GJB2* (gap junction B2) gene mutations are responsible for nearly 50% of the cases with autosomal recessive non-syndromic hearing loss. Fifty patients with hearing loss and 50 healthy volunteers were included in this study. Additionally, The parents having doubtful band pattern in the analysis of Single Strand Conformational Polymorphism (SSCP) were also included in this study. The ages of the subjects were between 15 and 35 years, and the study protocol was explained to all participants, and their approvals were taken. In 13 individuals with autosomal recessive non-syndromic hearing loss and with different band patterns by SSCP and PCR-RLFP techniques, the existence of W77X, V95M, 176-19del16, 167delT, 235delC and 35delG mutations has been also investigated. The purpose of this study is to scan *GJB2* mutations, having an influence on non-syndromic autosomal recessive hearing loss, in our region.

#### P12.047

#### Ryanodine receptor type 1 mutation analysis in malignant hyperthermia susceptibility type 1, central core disease, and multimicore disease.

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The ryanodine receptor type 1 (*RYR1*) gene encodes the skeletal-muscle ryanodine receptor that is fundamental in excitation-contraction coupling and calcium homeostasis. *RYR1* comprises 106 exons and encodes a protein of 5,038 amino acids. Mutations in *RYR1* are associated with malignant hyperthermia susceptibility type 1 (MHS1) and with core myopathies, including central core disease (CCD) and multimicore disease (MmD). The inheritance of these disorders is complex: MHS1 shows a dominant inheritance pattern, MmD a recessive inheritance pattern, while CCD can be inherited both in a recessive and in a dominant fashion. Additionally, maternal allele-silencing of *RYR1* may complicate this picture. MHS1 and CCD, though allelic disorders, do also share some specific mutations. To date, over 250 different pathogenic mutations are known, of which less than 8% clearly is an inactivating (nonsense/splice/frame-shift) mutation.

Recently, we have implemented sequence analysis of all 106 exons of *RYR1* and its splice sites in MHS, CCD and MmD. We have identified 16 likely-pathogenic alleles, of which two clearly are inactivating. Of these 16 alleles, 11 are novel mutations, and 1 allele harboring three missense mutations has been found in four families not known to be related. In one CCD patient, a mutation well known in MHS1 (p.Arg614Cys) and a splice-site mutation (c.14364+1G>T) were observed in trans orientation. These data indicate that mutations specific to the Dutch population exist. The complexity of the inheritance and

the obvious reduced penetrance of MHS make the determination of the pathogenicity of missense mutations and the subsequent counseling of patients challenging.

#### P12.048

#### A novel SMC1A gene mutation in a male with mild Cornelia de Lange syndrome

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Cornelia de Lange Syndrome (CdLS) (OMIM 122470 and 300590) is an inherited multisystem developmental disorder characterized by distinctive dysmorphic craniofacial features, growth and cognitive impairment and limb malformations. Since 2004, mutations in three genes (*NIPBL*, *SMC1A* and *SMC3*) of the cohesin complex and its regulators have been found in affected patients. To date, 11 different mutations in 14 unrelated patients have been reported in the X-linked *SMC1A* gene. Here, we identified a novel sporadic *SMC1A* mutation (p.R711Q) in a 14-month-old male who had characteristic but mild facial CdLS appearance, microcephaly, postnatal growth retardation, mild to moderate psychomotor delay, mild congenital heart defect (ASD and PDA) and 2-3 syndactyly in the feet. The mutation altered a highly conserved residue and was not detected in his parents or in 50 control individuals. It was located at the SMC coiled-coil domain and we hypothesize that it may affect its angulation within the protein. This case supports previously published work reporting that patients with mutations in the *SMC1A* gene show a milder physical phenotype, generally without severe limb malformations, than patients with mutations in the autosomal *NIPBL* gene.

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#### P12.049

#### Mutation spectrum of CYBB gene in Russian families with X-linked chronic granulomatous disease.

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Chronic granulomatous disease (CGD) is genetically heterogeneous immunodeficiency disorder characterized by a defect of intracellular bacterial killing in phagocytes due to reducing of activity of NADPH oxidase complex. CGD is characterized by recurrent life-threatening bacterial and fungal infections, granuloma formation. The most common form of chronic granulomatous disease is caused by mutations in the *CYBB* gene accounting for about 70% of all CGD cases. Located on chromosome Xp21.1 *CYBB* gene encodes the β-subunit of cytochrome b558(gp91-phox), spans 30 kb, contains 13 exons.

We have investigated 12 unrelated families with Chronic granulomatous disease. The search for *CYBB* gene mutations was performed by direct DNA sequencing analysis.

The analysis of *CYBB* gene showed 7 new and 3 reported mutations on 12 chromosomes. The *CYBB* gene total deletion was registered on 2 chromosomes, novel c.27delG - on 1 chromosome, novel c.226delC - on 2 chromosomes, novel c.177C>A (p.Cys59Stop) - on 1 chromosome, novel c.565\_568delATT - on 1 chromosome, c.676C>T (p.Arg226Stop) - on 1 chromosome, c.935T>G (p.Met312Arg) - on 1 chromosome, novel c.1167delG - on 1 chromosome, novel c.1524\_1527delGACT - on 1 chromosome, novel c.898\_1ntG>A (IVS8as-1ntG>A) - on 1 chromosome. The deletion c.27delG was de novo mutation: the patient's mother was not heterozygous for it.

10 *CYBB* mutations have been registered in this investigation. Most mutations were distributed throughout the 13 exons or at exon-intron boundaries, 6 of these mutations were unique. According to obtained data we concluded that the direct DNA sequencing analysis is the best method for diagnostics for Chronic granulomatous disease.

## P12.050

## CYP21A2 gene mutations in Portuguese CAH patients

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Congenital adrenal hyperplasia (CAH) is a common inherited autosomal recessive disorder of adrenal hormone biosynthesis due to mutations in the *CYP21A2* gene, which encodes the enzyme 21-hydroxylase. CAH is responsible for different degree of virilisation of external genitalia in newborn girls and, in its most severe form, can be fatal if not treated adequately. Patients with the mild, nonclassic form of the disease have less severe symptoms, associated with signs of postnatal androgen excess. Genotyping for ten of the most frequent mutations was performed in 84 Portuguese CAH patients; 10 salt-wasters, 5 simple-virilizers and 69 non-classical patients. The patients were diagnosed by a dosage of 17-hydroxyprogesterone above 10 ng/ml either in basal conditions or after an ACTH 0,25mg IV Test. A variety of genotyping techniques were utilized to detect these ten mutations. *CYP21* mutations were detected in 91.7% (77/84) of the patients. Among CAH patients, 9.5% presented two or more *CYP21* mutations. The most frequent mutations identified in our population were V281L (41.7%) and deletions/conversions involving the promoter region of the *CYP21* gene (28.3%). A decreased frequency of IVS2-12C/A>G mutation (5.6%) was the most characteristic feature of this population, suggesting some particularities of the Portuguese population regarding the mutations frequency.

## P12.051

## Investigation of genetic aspects of congenital and early childhood deafness in special school for children with impaired hearing of Kirov region, Russia.

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Two special schools for children with deafness of Kirov region were studied. 94 children with severe hearing loss from Kirov school and 57 children with mild and moderate hearing loss from Sovietsk school were examined. The examination included ENT physician examination, genetic consultations, and determination of 35delG mutation in *GJB2* gene and two big deletions in *GJB6*-gene, 342-kb (*GJB6-D13S1854*) and 309-kb (*GJB6-D13S1830*). Non-syndromic deafness was diagnosed in 140 cases; hereditary syndromal pathology was detected in 11 children. 35delG mutation in *GJB2* gene was discovered in 45 children (32.14%) with non-syndromic deafness (30 in homozygous state and 15 in heterozygous state). 342-kb and 309-kb deletions in *GJB6* gene were not detected. All the above patients with non-syndromic deafness were divided into 2 groups. Group 1 with a family history of deafness (28 children), and group 2 (112 children) without a family history of deafness. In group 1, 11 children (39.29%) had 35delG mutation in homozygous state, 3 had heterozygous state. Thus, the rate of 35delG mutation was 50%, severity of hearing impairment was severe and profound. In group 2, 35delG mutation was revealed in 25.0% of cases (18 in homozygous and 10 in heterozygous state). Of the above 18 homozygous patients, 6 patients had an additional combination of non-hereditary factors. Of the above 10 heterozygous patients, 6 patients had an additional combination of non-hereditary factors. The findings of the study show the necessity of DNA-diagnosis not only in case of a family history of deafness but also in non-hereditary factors.

## P12.052

## Deaf by fever!

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Auditory Neuropathy/ Auditory Dyssynchrony (AN/AD) is an hearing impairment characterized by severely distorted or absent brainstem evoked potential caused by an abnormal transmission of the auditory signal to the brainstem and preserved otoacoustic emissions due to normal function of the outer hair cells. Various genetic and non genetic aetiologies of AN have been identified but the vast majority of the cases are still unexplained. A worsening of the hearing defect concomitant with fever has been reported in few cases. The causes of this temperature dependent auditory neuropathy are unknown.

We report a consanguineous family with three siblings affected by a temperature dependent auditory neuropathy. The patients (10, 9 and 7 years old) had normal hearing to mild hearing impairment with normal otoacoustic emissions and impaired auditory brainstem responses. The family history describes transient hearing loss associated with fever episodes. Imaging did not find any cochlear nerves or brain abnormalities. The family genotype was consistent with linkage to the DFNB9/OTOF region. Molecular analysis of the 48 exons and intron-exon boundaries of OTOF reveals a novel mutation. The mutation p.Glu1803del, in exon 44 of OTOF was found homozygous in the patients and segregates with the hearing impairment in the family. Otoferlin is a protein expressed in the inner hair cells and is essential for the synaptic vesicle fusion. The new mutation described here, is located in the C2F otoferlin domain and is associated with an unusual phenotype compared to previous reported cases of patient affected by OTOF mutations.

## P12.053

## DFNB1 locus mutation analysis in Latvian patients with nonsyndromic sensorineural hearing loss

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**Background:** Hearing loss is the most common birth defect and the most prevalent sensorineural disorder in developed countries. More than half of prelingual deafness cases are due to genetic factors. About 70% of all hereditary deafness cases are classified as nonsyndromic and recessive.

Approximately 50% of autosomal recessive nonsyndromic hearing loss can be attributed to the disorder DFNB1, caused by mutations in the genes *GJB2* and *GJB6* (which encode proteins connexin 26 and connexin 30). DFNB1 has digenic pattern of inheritance. The 35delG mutation in *GJB2* gene is the most common mutation in DFNB1 in many populations.

**Materials:** We obtained 151 DNA samples from patients with prelingual hearing loss in whom syndromic forms and environmental causes of deafness had been excluded, their relatives and individuals with hearing loss positive family history.

**Methods:** DNA was extracted from whole blood. The *GJB2* exon 2 analysis was performed using PCR, enzymatic restriction and automated sequencing. Analysis of del(*GJB6-D13S1830*) and del(*GJB6-D13S1854*) in *GJB6* was performed by multiplex-PCR.

**Results:** 65 unrelated patients were screened for the *GJB2* mutations. Four different mutations in the *GJB2* gene have been identified in Latvian DFNB1 patients: 35delG, 311-324del14, 235delC and M34T. One heterozygous 51del12insA mutation was detected in unaffected individual with positive family history.

Two causative *GJB2* mutations were found in 37 patients (56%), 25 patients had no *GJB2* mutations, three patients are heterozygous for one *GJB2* mutation and the cause of impairment remains unclear.

We have started testing for two *GJB6* deletions and the results are in process.

## P12.054

## Identification of a novel mutation in RPS19 in a patient with Diamond-Blackfan anemia

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Diamond-Blackfan anemia (DBA) is a rare, pure red blood cell aplasia of childhood caused by an intrinsic defect in erythropoietic progenitors. Malformations occur in about 40% of patients. Mutations in the gene encoding ribosomal protein S19 (RPS19) are found in 25% of patients. We found a novel nonsense mutation in RPS19 in a patient with DBA. The proband was a 1 year old girl presented with severe anemia at birth. Transfusion dependence persisted into infancy. Anemia was hyporegenerative with absent reticulocytes and slight macrocytosis. Peripheral blood platelet and granulocyte count was within normal limits. Bone marrow aspiration showed absent erythroid series with normal granulocytic and megakaryocyte compartment. Bone marrow PCR analysis for parvovirus B19 was negative. The patient had no skeletal abnormalities. Her family history was unremarkable. The clinical diagnosis of congenital pure red cell aplasia was established. Genomic DNA amplification and sequencing of the coding exons and their flanking regions of the RPS19 gene revealed a nonsense mutation in exon 3 in heterozygous state: c.156 G>A (Trp52Stop). Two other mutations in the same codon have been previously described: c.154 T>C (Trp52Arg) and c.155 G>A (Trp52Stop) (Willig, Drapchinskaia, 1999), and our data are consistent with identified "a hot spot" for missense/nonsense mutations between codons 52-62, a highly conserved region likely to have a critical role in RPS19 function.

#### P12.055

#### Histological and molecular investigation of a dystrophic epidermolysis bullosa Tunisian family with phenotypic variability

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Dystrophic Epidermolysis Bullosa (DEB) is inherited in both autosomal dominant DDEB and autosomal recessive manner RDEB, both of which result from mutations in the type VII collagen gene (COL7A1), leading to the dermal-epidermal junction fragility. DEB is characterized by inter and intrafamilial phenotypic variability. We investigated, here, a large multiplex Tunisian family with five affected members: four affected by the pretibial DEB form and one by the generalized RDEB.

Indirect immunofluorescence (IF) with the antibody LH7:2 against collagen VII and electron microscopy (EM) analyses were performed. The members of the family were genotyped with five markers flanking COL7A1, and screening for the deleterious mutation by DHPLC and direct sequencing.

Molecular investigation showed that all family members, unaffected and affected by the pretibial form, were heterozygous for the c.7178delT mutation, except for the generalized RDEB member who was homozygous. Moreover, IF showed no correlation with the affected and the healthy status of the heterozygous individuals. These results are suggestive for an autosomal semidominant model of inheritance with incomplete penetrance and variable expression for the identified mutation. No genotype phenotype correlation was observed suggesting the existence of other genetic determinants influencing dermo-epidermal junction cohesion.

#### P12.056

#### Genetic investigation of Epidermolyticus verruciformis in four Tunisian patients

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Epidermolyticus verruciformis (EV; MIM 226400) is a rare genodermatosis that is clinically characterized by flat, wart-like and pityriasis versicolor-like lesions. The disease is associated with high risk of skin cancer. EV results of a genetically determined abnormal susceptibility to a specific oncogenic group of related human papillomavirus (HPV), usually HPV type 5.

Recently, homozygous mutations in either of two adjacent EVER1 or

EVER2 gene localized on chromosome 17q25 in EV1 locus have been identified to underline EV among 75% of patients with different ethnic origins.

In the present study, we report clinical, histological and molecular investigations of 4 EV Tunisian patients.

Clinical examination revealed three types of lesions among examined patients: flat warty, pityriasis versicolor-like macules and seborrheic like changes. Histological observations of EV lesions showed hyperkeratosis, hypergranulosis, acanthosis and papillomatosis. The keratinocytes are vacuolated and show a clear blue-grey pale cytoplasm and a central pyknotic nucleus. The clinical and histological findings showed no squamous cell carcinoma.

As for several genodermatoses, similar mutational spectrum has been identified among Algerian and Tunisian patients, EV Tunisian patients were screened for the "Algerian mutations" (280 C→T and 754 or 755 del T) within EVER1 and EVER2, respectively.

None of the two Algerian explored mutations was found, suggesting that Tunisian patients do not share the same mutations as those described among Algerians. Genotyping of HPV associated to EV in Tunisian patients will be identified in order to determine the etiology of the virus involving in the disease and to establish a possible phenotype-genotype correlation.

#### P12.057

#### Tyrosinemia type 1: identification of a novel mutation in two unrelated individuals

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Tyrosinemia type I results from deficiency of the enzyme fumarylacetate hydrolase (FAH), encoded by the FAH gene. This gene is located at 15q23-25 and contains 14 exons. Untreated tyrosinemia type I usually presents either in young infants with severe liver involvement or later in the first year with liver dysfunction and renal tubular dysfunction associated with growth failure and rickets. Tyrosinemia type I is inherited in an autosomal recessive manner. The four common FAH mutations (IVS12+5G>A, IVS6-1G>T, IVS7-6T>G, p.Pro261Leu) account for approximately 60% of mutations in the general US population.

Here we report two unrelated Iranian patients with a novel mutation in the FAH gene. The whole FAH gene of the patients were screened by direct sequencing and it was established that mutation is: c.709 C to T (R237X) at exon 9 of the gene in homozygous form. This mutation leads to a premature stop codon (R237X), and can be considered disease causing.

So far limited number of mutations in the FAH gene have been reported in type I tyrosinemia patients. Most of them are single-nucleotide substitutions occurring in the splice sites. Most of the mutations in FAH gene occur in a particular region between residues 230 and 250. This area seems to be a hotspot region within the gene. The mutation identified in this study also resides within this hotspot. This novel mutation was shared by two unrelated individuals inhabiting different locations in Iran. As a result, we assume it might be a common mutation in our population.

#### P12.058

#### Spectrum of LDLR mutations and role of PCSK9 as a modifier gene in familiar hypercholesterolemia in Lebanon

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Autosomal dominant hypercholesterolemia (ADH), a major risk for coronary heart disease, is associated with mutations in the genes encoding the low-density lipoproteins receptor (LDLR), its ligand apolipoprotein B, and PCSK9 (Proprotein Convertase Subtilisin Kexin 9), the 3<sup>rd</sup> gene that we identified in the disease in 2003. Familial hypercholesterolemia (FH) caused by mutation in the LDLR gene is the most frequent form of ADH. The identification of genes that modify the phenotype of FH is very difficult because more than 1000 LDLR mutations

have been reported worldwide with different impact on the severity of the disease.

In this study, we characterize the spectrum of the mutations causing FH in Lebanon, where the incidence of FH is particularly high, presumably as a result of a founder effect. We confirm the very high frequency of the *LDLR* p.Cys681X mutation that accounts for 81.5 % of the FH Lebanese probands recruited and identify other less frequent mutations in the *LDLR*. Finally, we show that the p.Leu21dup, an in frame insertion of one leucine to the stretch of 9 leucines in exon 1 of *PCSK9*, known to be associated with lower LDL-cholesterol levels in general populations, is also associated with a reduction of LDL-cholesterol levels in FH patients sharing the p.C681X mutation in the *LDLR*. Thus, by studying for the first time the impact of *PCSK9* polymorphism on LDL-cholesterol levels of FH patients carrying a same *LDLR* mutation, we show that *PCSK9* might constitute a modifier gene in familial hypercholesterolemia.

#### P12.059

#### Heterozygous mutation in MEFV have a potential triallelic effect on patients with two mutations in MVK gene?

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**Background:** Familial Mediterranean Fever (FMF) is an autosomal recessive disorder characterized by fever and synovial inflammation. FMF is caused by mutations affecting both alleles of MEFV gene. Hyper-IgD (HIDS) syndrome is an autosomal recessive disorder characterized by recurrent episodes of fever associated with lymphadenopathy, arthralgia, gastrointestinal disturbance, and skin rash. HIDS is caused by mutations of MVK gene.

**Patients and Methods:** In our study, we describe a Sicilian (ITALY) patient with typical symptoms of the FMF. Moreover, he was affected by an unusual type of HIDS (IgD not detectable). Interesting, the proband was heterozygote for a mutation of MEFV gene (V726A) and he was also compound heterozygote for two mutations of MVK gene (V377I, P228L). The P228L, here described for the first time, is a novel missense mutation involving the exchange of a proline (CCA), highly conserved in mammals and birds, with a Leucine (CTA).

**Conclusions:** The findings of this study encourage our assumptions about the triallelic transmission of the syndromes associated with periodic fevers, on the basis of the identification of 3 mutated alleles in 2 different genes. The third mutation in the gene MEFV could play an epistatic role, modifying the spectrum symptoms of HIDS. Our data suggest that the study of a HIDS and FMF wider population would be important not only to clarify the genetic heterogeneity of this group of syndromes, but mainly to establish a new diagnostic and therapeutic approach to these patients.

#### P12.060

#### Novel FBP1 gene mutations in Arab patients with fructose-1,6-bisphosphatase deficiency.

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Deficiency of fructose-1,6-bisphosphatase (FBP) results in impaired gluconeogenesis, which is characterized by episodes of hyperventilation, apnea, hypoglycemia, and metabolic and lactic acidosis. This autosomal recessive disorder is caused by mutations in the FBP1 gene, which encodes for fructose-1,6-bisphosphatase 1 (FBP1). Although FBP1 gene mutations have been described in FBP deficient individuals of various ethnicities, there has been limited investigation into the genetics of this disorder in Arab patients. This study employed five consanguineous Arab families, in which seventeen patients were clinically diagnosed with FBP deficiency. Seven patients and six carrier parents were analyzed for mutations in the FBP1 gene. DNA sequencing of the FBP1 gene identified two novel mutations in these families. A novel six nucleotide repetitive insertion, c114\_119dupCTGCAC, was identified in patients from three families. This mutation encodes for a duplication of two amino acids (p.Cys39\_Thr40dup) in the N-terminal domain of FBP1. A novel nonsense c.841G>T mutation encoding for

a p.Glu281X truncation in the active site of FBP1 was discovered in patients from two families. The newly identified mutations in the FBP1 gene are predicted to produce FBP1 deficiency. These mutations are the only known genetic causes of FBP deficiency in Arab patients. The p.Cys39\_Thr40dup is the first reported amino acid duplication in FBP deficiency patients. We conclude that this study provides a strong rationale for genetic testing of FBP deficient patients of Arab ethnicity for recurrent or novel mutations in the FBP1 gene.

#### P12.061

#### Association of the neonatal Fc receptor gene VNTR polymorphism with primary defects of antibody production

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**Introduction:** The neonatal Fc receptor (FcRn) for IgG has been characterized in the transfer of passive humoral immunity from mother to fetus. It transports IgG from maternal circulation to the fetal capillaries of the placenta villi. Moreover, FcRn protects IgG from degradation and thus prolongs a half-life of IgG antibodies in the serum. A variable number of tandem repeats (VNTR) polymorphism in the promoter region of the *FcRn* gene has recently been described. Allele \*2 showed decreased promoter activity resulting in lower expression and decreased monocyte binding capacity for IgG compared to wild type allele \*3.

**Material and methods:** We analysed a distribution of the *FcRn* gene VNTR polymorphism in 202 samples of general Czech population,

61 common variable immunodeficiency (CVID) patients, 21 X-linked agammaglobulinemia (XLA) patients, and 5 individuals with low IgG

but normal IgA levels, using PCR. Fisher exact test was used for statistical analysis.

**Results and discussion:** The allele \*2 was significantly more frequent in patients with primary hypogammaglobulinemia compared to general population (11.5% v.s. 6.2%, p=0.02). While its frequency in CVID patients did not differ from controls (8.2%, p=0.28), significantly more carriers of this allele were detected among XLA patients and patients with low IgG and normal IgA levels (16.7%, p=0.02, and 30.0%, p=0.02, respectively). More data are needed to better characterize a potential role of the *FcRn* gene VNTR polymorphism in disease manifestation in particular subgroups of patients with primary defects of antibody production.

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#### P12.062

#### Congenital factor XIII deficiency caused by the same mutation in five Tunisian families

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Inherited factor XIII (FXIII) deficiency is a rare bleeding disorder that can present with umbilical bleeding during the neonatal period, delayed soft tissue bruising, mucosal bleeding spontaneous intracranial hemorrhage and soft tissue hemorrhages. Congenital FXIII deficiency is an autosomal recessive disorder, usually attributed to a defect in the FXIII A and B subunits coding by F13A and F13B genes respectively. The aim of this study was to determine the molecular defects responsible of congenital deficiency of factor XIII in five Tunisian families.

Our diagnoses included antigen determination of FXIII A and B subunits in both plasma and platelets. Molecular analysis was performed by direct DNA sequencing of polymerase chain reaction amplified fragments spanning the coding regions and splice junctions of the FXIII A subunit gene (F13A) in probands and in families' members and compared with the reported sequence of this gene.

The measurement of the A and the B antigen levels showed that, in all cases, FXIII A was undetectable and FXIII B was within the normal range. Direct sequencing of the F13A gene for all probands showed the same "c.869 insC" mutation. This is a frame shift mutation leading to a null allele. In addition to this mutation three new polymorphisms had been identified.

We describe here the molecular abnormality found in five Tunisian pro-

bands diagnosed with FXIII deficiency. The identification of this founder mutation and polymorphisms allowed a genetic counselling in relatives of these families and the antenatal diagnosis is now available.

#### P12.063

#### A survey of PORCN mutations in focal dermal hypoplasia - strategies to survive lethal mutations

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Focal dermal hypoplasia (FDH, Goltz syndrome, MIM #305600) is a pleiotropic birth defect characterized by widespread lesions of dermal hypoplasia or even aplasia. The resulting skin changes follow the lines of Blaschko, indicating mosaicism. Another major diagnostic sign is longitudinal striation of the long bones, likewise hinting to functional mosaicism. Associated variable features include areas of hairlessness, hypoplasia or aplasia of bones, malformations of the autopod, and microphthalmia or unilateral anophthalmia. In addition, hypodontia, hearing loss, horseshoe kidney, and papillomatosis of the larynx may be found.

Recently, we had shown that the disease is caused by mutations of PORCN in Xp11.23 (Nature Genetics 2007, 39: 833-835). The attachment of palmitoleic acid by the O-acyltransferase PORCN prepares Wnt signaling molecules for the transport through the Golgi apparatus for secretion, signal gradient formation, and receptor binding.

We have replenished the scope of known mutations considerably by analyzing PORCN in 24 novel patients:

- i) In sporadic cases PORCN is affected primarily by nonsense mutations whereas large deletions encompassing various neighboring genes are mostly familial.
- ii) The missense mutations known so far almost exclusively exchange highly conserved amino acids in transmembrane domains or in the luminal loops.
- iii) To override the consequences of lethal PORCN mutations, male patients are somatic mosaics or show more than one X-chromosome. Females, survive either due to extreme skewing of X-chromosome inactivation, which is particularly evident in familial deletions, or they appear to be likewise somatic mosaics.

#### P12.064

#### Evaluation of the Qiaxcel system for the analysis of products generated with the Abbott Fragile-X PCR kit

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Fragile X syndrome is the single most common inherited cause of mental impairment and shows an approximate prevalence 1/4000. The gene responsible for fragile X syndrome, *FMR1*, contains an unstable repeat sequence of (CGG)<sub>n</sub> that when expanded causes the syndrome. PCR is a fast and effective method for the diagnosis of (CGG)<sub>n</sub> repeat number. However, PCR methods traditionally were only useful for repeat sizes in the lower premutation ranges (up to 70-100 repeats) because amplification becomes increasingly difficult as repeat number increases. Recently, a commercial kit by Abbott has become available that has overcome such problem, as it amplifies products in all the premutation ranges (up to 200 repeats) and full mutation ranges (over 250 repeats). The analysis and sizing of the fluorescence labelled products generated using the Abbott fragile-X kit require a genetic analyser (sequencer). This equipment is expensive and not all laboratories have access to one. As an alternative, we have successfully tested the Qiaxcel system of Qiagen. We have found such a system adequate for routine screening purposes with the Abbott Fragile-X PCR kit. However, samples that are in the higher resolution limit (full mutations) or lower resolution limit (females with 1 repeat differences), need to be analysed under special conditions. The removal of the gender primers increases the PCR efficiency for large repeat sizes and may be more adequate for the detection of full mutations.

#### P12.065

#### Friedreich Ataxia and Mitochondria

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Friedreich's Ataxia (FA) is the commonest genetic cause of ataxia and is associated with the expansion of a GAA repeat in intron 1 of the frataxin gene. Frataxin deficiency leads to excessive free radical production, dysfunction of respiratory chain complexes and progressive iron accumulation in mitochondria. Common deletion were identified and confirmed by southern blotting in FA patients. Homozygous GAA expansion was found in 21 (84%) of all cases. In four cases (16%), no expansion was observed, ruling out the diagnosis of Friedreich's ataxia. In cases with GAA expansions, ataxia, scoliosis and pes cavus, cardiac abnormalities and some neurological findings occurred more frequently than in our patients without GAA expansion. Molecular analysis was imperative for diagnosis of Friedreich's ataxia, not only for typical cases, but also for atypical ones. mtDNA deletions were present in 76% of our patients representing mtDNA damage, which may be due to iron accumulation in mitochondria. Our findings showed that complex I activities and intracellular ATP were significantly reduced ( $P=0.001$ ) in patients compared with control. 8.6 kb deletion in mtDNA was detected in all of patients by multiplex PCR but Southern blot analysis confirmed the presence of deletion in 9 of 12 patients. Decreased Frataxin expression in FA cells result in accumulation of mitochondrial iron and increased free iron levels leads to free radical generation, increasing mtDNA mutation and decreasing complex I activity and intracellular ATP content.

#### P12.066

#### Identification of novel mutations of the mitochondrial DNA associated with Iranian Friedreich's ataxia

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Friedreich's ataxia (FRDA) is an autosomal recessive neurodegenerative disorder caused by decreased expression of the protein Frataxin. Frataxin deficiency leads to excessive free radical production. Mitochondrial DNA (mtDNA) could be considered a candidate modifier factor for FRDA disease. It prompted us to focus on the mtDNA and monitor the nucleotide changes of genome which are probably the cause of respiratory chain defects and reduced ATP generation. We searched about 46% of the entire mitochondrial genome by Temporal Temperature Gradient Gel Electrophoresis (TTGE) and DNA fragments showing abnormal banding patterns were sequenced for identification of the exact mutations.

In 20 patients, for the first time we detected 26 Mitochondrial DNA mutations which 11 (42.5%) was novel and 15 (57%) have been reported in other diseases. Our results showed that NADH dehydrogenase (ND) genes mutations in FRDA samples was higher than normal controls ( $P<0.001$ ) and we found statistically significant inverse correlation ( $r = -0.77$ ) between number of mutation in DN genes and age of onset in FRDA patients. It is possible that mutations in ND genes could constitute a predisposing factor that in combination with environmental risk factors affect on age of onset and disease progression.

#### P12.067

#### Chaperone effect of several iminosugars and aminocyclitols on mutated glucocerebrosidases: a possible therapeutic approach for Gaucher disease

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de Biología, Universitat de Barcelona, CIBERER, IBUB, Barcelona, Spain. Gaucher disease is an autosomal recessive disorder. It is characterized by the accumulation of glucosylceramide in lysosomes of the mononuclear phagocyte system, attributable to glucocerebrosidase (GBA; EC3.2.1.45) deficiency. The main consequences of this disease are hepatosplenomegaly, skeletal lesions and, sometimes, neurological manifestations. It has been shown that at sub-inhibitory concentrations, several competitive inhibitors act as chemical chaperones by inducing protein stabilization and increasing enzymatic activity. We have tested the chaperone effect of two iminosugars, *N*-(n-nonyl)-deoxyojirimycin (NN-DNJ) and *N*-(n-butyl)-deoxyojirimycin (NB-DNJ), and four aminocyclitols with distinct degrees of lipophilicity on mutated GBAs. The analyses were performed on COS-7 cells transfected with ten different mutant GBA cDNAs and on patient fibroblasts with diverse genotypes. We have shown an increase in the activity of GBA with NN-DNJ, NB-DNJ and aminocyclitol 1 in stably transfected cell lines for some of the mutations and with NN-DNJ and aminocyclitol 4 in some of the patients' fibroblasts. These promising results on specific mutations validate the use of chemical chaperones as a therapeutic approach for Gaucher disease. However, the development and analysis of new compounds is required. As a next step, we are currently analysing the abnormal intracellular trafficking of mutant glucocerebrosidases by immunohistochemical techniques and confocal microscopy. The cells are being treated with the compounds that showed to have a chaperone effect (NN-DNJ, NB-DNJ, aminocyclitol 1 and aminocyclitol 4), in order to analyse if the enzymes from treated cells can reach the lysosomes.

#### P12.068

#### Generalized Atrophic Benign Epidermolysis Bullosa (GABEB), caused by a new COL17A1 truncation mutation in a Tunisian patient

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Generalized Atrophic Benign Epidermolysis Bullosa, GABEB (OMIM# 226650), is a nonlethal variant of junctional epidermolysis bullosa with autosomal recessive inheritance pattern. Clinically, it is characterized by blistering of the skin since birth, atrophy of the affected skin, dystrophic nails, dental anomalies and alopecia. The pathogenesis of this disorder is generally caused by mutations affecting the *BPAG2/COL17A1* gene encoding hemidesmosomal transmembrane protein; the 180 KDa bullous pemphigoid antigen (BP180), also known as type XVII collagen. In this study we describe a Tunisian GABEB patient clinically EB affected who showed an absence of expression of BP180 at the dermal-epidermal junction revealed by immunohistochemical staining analyses using antibodies against the type XVII collagen. The *COL17A1* gene sequencing has revealed a homozygous C458X mutation in exon 17 of this gene responsible of the disease. The parents' sequencing shows a heterozygous state of this mutation.

#### P12.069

#### GJB2 mutations in Macedonian patients with non-syndromic hearing loss

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Hearing impairment is one of the most common sensory-neural disorders with the incidence of profound deafness in one per 1,000 births. Mutations in the GJB2 and GJB6 genes for DFNB1 locus (13q12) are responsible for about half of all cases of autosomal recessive prelingual hearing loss. Among them 35delG mutation accounts for approximately 70% of all GJB2 mutant alleles in most European populations. The aim of the study was to evaluate the frequency and type of mutations in GJB2 gene, as well as the frequency of GJB6 deletion among

Macedonian patients with non-syndromic hearing loss (NSHL). We have analyzed 33 patients with prelingual NSHL.

SSCP analysis following by direct sequencing in fragments where altered electrophoretic mobility was assigned was used for detection of mutations in GJB2 gene, while specific PCR using two sets of primers were used for (GJB6-D13S1830)del screening.

In 12 out of 33 patients (36.4%) mutations in GJB2 gene were found. Among 22 mutated chromosomes, 15 (68.2%) carried 35delG mutation. Other common mutations Trp24Stop, Val37Ile and Arg127His, with a frequency of 6.06%, 3.0% and 1.5%, respectively, were found. (GJB6-D13S1830)del mutation was not found in our group of patients.

Since high mutation rate was observed in GJB2 gene in NSHL patients, testing should be performed as a routine screening in cases with prelingual deafness.

#### P12.070

#### Screening of mutations in MYOC, CYP1B1 and OPTN in Spanish families with glaucoma and ocular hypertension

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Glaucoma is a heterogeneous group of optic neuropathies and represents the second most prevalent cause of blindness worldwide, projected to affect more than 60 million people by 2010. The purpose of this study was the identification of MYOC, CYP1B1 and OPTN mutations in a Spanish population affected by different clinical forms of familial glaucoma or ocular hypertension, in order to determine the best therapeutic approach.

Clinical studies were performed with ophthalmologic examination that included pachymetry-corrected intraocular pressure applanation tonometry and optical coherence tomography or Heidelberg retinal tomography. Mutation detection was performed in 202 individuals from 84 families with a positive family history of glaucoma. Screening of mutations in the MYOC gene in index patients revealed three previously reported mutations (Gln368Stop, Val426Phe and Ala427Thr) and one novel mutation (Glu218Lys). We performed CYP1B1 mutation analysis in index patients affected by primary congenital glaucoma, and six previously reported mutations were detected, namely Gly61Glu, His-354fs, Arg368His, Thr403fs, Asp449fs and Arg469Trp. OPTN mutation screening is currently being performed and up to now we have not found any mutation associated to glaucoma.

We also present phenotype-genotype correlation in relatives of patients with mutation in any of the analyzed genes. The genetic analysis helps us to provide a more accurate visual prognosis as well as appropriate genetic counselling.

#### P12.071

#### Molecular, hematological aspects in HbH disease

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Background: H disease is caused by deletion or inactivation of three alpha-globin genes. People with H disease usually have moderate anaemia, but are generally thought to be asymptomatic. Some H disease patients require transfusions, and there are non deletional forms of Hb H. Here we describe hematologic feature of cases with Hb H disease.

Material and Methods: In this study we have defined the molecular basis and the clinical phenotype with the alpha-globin genotype in 18 Iranian patients with HbH disease. HbH disease was diagnosed according to abnormal red cell morphology including hypochromia, HbH inclusion and persistence of HbH in Hb electrophoresis.

Result: The most molecular defect was the deletional most commonly the (-/-alpha 3.7) genotype. In this study, 15 were deletional forms of Hb H (-/-alpha) who had not received any blood transfusion and three patients had the nondeletional type of hemoglobin H disease (-/alpha alpha<sup>T</sup>) with transfusion-dependent Hb H disease. Mean MCV and MCH for deletional were  $58.04 \pm 4.31$  fl and  $17.5 \pm 1.2$  pg respectively (range from 46.6 to 64.9 for MCV and 14.3 to 19.6 for MCH). HbH was elevated significantly in all cases (mean value  $9.3 \pm 5\%$  ranging from 1.2-19.6%). HbH levels are usually higher in non deletional than in deletional, ( $P$  value <0.0001). A significant correlation was found between MCV values and genotype, ( $P$  < 0.0001).

Conclusion: The (–/alpha alpha<sup>T</sup>) genotype has more severe disease than those with the (–/–alpha) and the most molecular defect was (–/–alpha 3.7).

Key words: H disease, Hb H, genotype

#### P12.072

##### Deletion of the HFE gene is present at the population level in Sardinia

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Introduction: Very recently, we reported the case of a woman of Sardinian descent who had a major structural alteration in the HFE gene. Molecular characterization revealed an Alu-mediated recombination causing the loss of the complete HFE gene sequence. Although homozygous for the HFE deleted allele, the woman had a phenotype similar to that seen in most women homozygous for the common p.C282Y mutation. The deletion was not detected in a cohort of iron overload patients of Northern European descent. Here, we focused on DNA from Sardinia patients.

Methods: We looked for the HFE deletion by using a rearrangement specific PCR. Positive results were confirmed by QFM-PCR and sequencing.

Results: The HFE deleted allele was detected in two of 24 unrelated patients. Both patients were previously viewed as homozygous for the common p.H63D variation. At diagnosis, they presented with moderate iron overloads.

Conclusion/Discussion: Deletion of the complete HFE gene sequence is not private, but present at the population level in Sardinia. Additional studies have been started to ascertain the assumption of a founder effect and precisely investigate frequency of the HFE deleted allele. In future, the recognition of several individuals with the HFE deletion will provide another opportunity to better understand penetrance of the common p.C282Y/p.C282Y genotype, which remains a matter of debates.

#### P12.073

##### The IVSI-5 (G>C) β-thalassemia mutation in trans with the (delta) CD12 (AAT>AAA) Hb A<sub>2</sub> NYU in an Iranian family

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Beta-thalassemia is the most common hereditary disorder in Iran. The ability to premarital screening for thalassemia is in effect in Iran since 1997. Typical heterozygous carriers of β-thalassemia have Hb A<sub>2</sub> >3.5, however, in some carriers of it is in normal range. Here we report the co-inheritance of β- and δ-globin gene mutations in an individual with microcytosis, hypochromia and normal Hb A<sub>2</sub>. Among couples referred to our lab from Primary Health Care centers (PHC) for molecular testing and prenatal diagnosis of β-thalassemia in Tehran, one individual was found to be atypical to be investigated for β-thalassemia. After obtaining informed consent, CBC and Hb electrophoresis was performed and genomic DNA was extracted from peripheral blood Leukocytes by salting out method. Amplification refractory mutation system PCR (ARMS-PCR) and direct DNA sequencing of δ-globin gene was exploited for detection of beta and delta globin gene mutations. The CBC and Hb electrophoresis pattern showed low indices and normal Hb A<sub>2</sub>. ARMS-PCR technique for β-globin gene mutation revealed the beta<sup>0</sup> IVS-I-5 (G to C) mutation. Direct DNA sequencing of δ-globin gene and hematological studies of the family members confirmed the presence of mutation in delta codon 12 (AAT > AAA). We suggested this individual carries a delta globin gene mutation which is unable to increase the delta-globin chain output in response to beta-thalassemia. Direct DNA sequencing confirmed the co-inheritance of mutation in δ-globin gene as being codon 12.

#### P12.074

##### Identification of a new β-globin variant (β133 GTG>ATG) in a family from Messina (Sicily-Italy)

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The hemoglobinopathies, or structural Hb variants, are attributable to aminoacid substitution in either the α or non-α chain. We studied five members of a family from Messina (Sicily-Italy). The proband, a 39 years old male, was investigated because he showed a little decrease in MCV values (79 fl) and a slightly increased HbA<sub>2</sub> levels (3.5%). Molecular analysis by directly sequencing of the amplified β globin gene revealed the presence of two single point mutations, a C>G transition at codon 70 (GCC>GGC) and a G>A base substitution at codon 133 (GTG>ATG). Therefore, we examined 5 members belonging to three generations of the family. The new variant, called Hb Messina, (β133 Val>Met GTG>ATG) was identified in heterozygosis in four subjects. Moreover, Hb Hershey was identified in heterozygosis in one subject (III.2). Familiar analysis showed that G>A base substitution at codon 133 was inherited from the father (I.1). Hb Messina shows asymptomatic clinical phenotype in heterozygotes subjects (I.I, II.3, II.4 and III.1); however, two family members had slightly increased HbA<sub>2</sub> levels (3.5-3.6 %) and showed a little decrease in MCV values (78-79 fl). The molecular analysis of the globin genes is important to perform an accurate diagnosis in the subjects with slightly alterations of the hematological parameters.

#### P12.075

##### Genetic analysis of 31 Iranian families segregating autosomal recessive hearing impairment

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Autosomal recessive non-syndromic hearing impairment (ARNSHI) is the most common form of monogenic hearing impairment. It is a highly genetic heterogeneous disorder, as up to now 60 loci have been mapped for ARNSHI. Iran, with a high rate of consanguineous marriage is a good genetic resource for studying ARNSHI. The aim of our project is to identify novel genes or mutations for ARNSHI. A set of Iranian families suffering from ARNSHI were studied. DNA sequencing of the coding exon of GJB2, as well as linkage analysis of the DFNB1 locus was performed and linked families were excluded from further analysis. Seventeen out of 31 remaining families had S-link LOD scores higher than the threshold value for genome-wide significance of 3.3. Linkage analysis of the 14 most common ARNSHI loci was performed for all the families. To confirm linkage, further markers were genotyped in the linked families. Eight families showed linkage to 4 different loci: 4 families to DFNB4 (SLC26A4), 1 to DFNB7/11 (TMC1), 1 to DFNB9 (OTOF), 1 to DFNB2 (MYO7A) and 1 to DFNB21 (TECTA). Mutation screening of these 4 known genes is being performed in the linked families. Four SLC26A4 mutations have already been found in the DFNB4 families. The results of this study support the notion that DFNB4 ranks second after GJB2 as a cause for ARNSHI. Twelve families with S-link LOD scores higher than 3.3 not linked to any of the 14 known loci will be included into genome-wide linkage analysis studies.

#### P12.076

##### Phenotype and genotype in females with pou3f4 mutations

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X-linked deafness is a rare cause of hereditary isolated hearing impairment estimated as at least 1 or 2% of the non-syndromic hearing loss. To date 4 loci for DFN have been identified and only one gene, POU3F4 responsible for DFN3, has been cloned. In males, DFN3 is characterized by either a progressive deafness associated with peri-

lymphatic gusher at stapes surgery and with a characteristic inner ear malformation. We have defined the phenotype of 8 independent females carrying *POU3F4* anomalies. A late-onset hearing loss is found in 3 patients. Only one has an inner ear malformation. No genotype/phenotype correlation is identified.

#### P12.077

#### An autosomal recessive nonsyndromic deafness locus is assigned to chromosome 18q12.3-21.1

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We studied a large non-consanguineous Ashkenazi family in which 5 of 10 children suffer from profound, prelingual hearing loss. A genome wide search mapped the disease gene to a n 8 Mb interval on chromosome 18q12.3-21.1, and a maximum lod score of 3.03 was obtained with the marker AC021763 at  $\theta = 0.00$ . Saturation of the region with additional polymorphic markers and SNP's revealed a 3.5 Mb homozygous interval in the affected sibs. The region contains 12 known genes, none of which have been previously associated with hearing loss. Sequencing of one gene from the interval, *SLC14A1* did not reveal any pathogenic variants in the exons or in the flanking intronic sequences. Currently additional genes are being sequenced. These results define a novel locus for autosomal recessive hearing loss on chromosome 18q12.3-21.1.

#### P12.078

#### High-resolution breakpoint mapping of novel rearrangements involved in alpha- and beta-thalassemia using array-Comparative Genomic Hybridization (aCGH)

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Thalassemias are hereditary microcytic hypochromic anemias characterized by abnormalities in hemoglobin production due to reduced expression of either the beta-globin gene, leading to beta-thalassemia, or the alpha-globin genes, giving rise to alpha-thalassemia. About 10% of the beta-thalassemias and 90% of the alpha-thalassemias are caused by deletions in either globin gene cluster. In a previous study, we applied Multiplex Ligation-dependent Probe Amplification (MLPA) to characterize large rearrangements in the alpha- and beta-globin gene cluster. Several new deletions and duplications were found, however, the exact breakpoint sequences are still unknown. To facilitate confirmation by breakpoint PCR and to gain more insight in the mechanisms causing these rearrangements we decided to determine the precise location of breakpoints.

Array Comparative Genomic Hybridization (aCGH) measures DNA copy number differences between a reference and a patient's genome sample thereby detecting and mapping deletions and duplications. We used high resolution tiling arrays with 135,000 probes spaced at a density of ~15 bp to map the breakpoints to an interval that can be validated by PCR and sequencing. The array was hybridized to a set of 55 thalassemia patients who were found to carry a deletion in the alpha- or beta-globin gene cluster. The fine mapping results were used to design breakpoint PCRs and resulting fragments were sequenced to determine the precise breakpoint.

#### P12.079

#### Multiple ligation-probe dependent amplification (MLPA) and Hemophilia A: detection of deletions in patients and tool for carrier status in female relatives.

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Haemophilia A is an X-linked bleeding disorder caused by mutations widespread in the human coagulation F8 gene. Most of the mutations in the F8 gene are detectable using genomic sequencing analysis. However, deletions of one or more exons or encompassing the entire gene can go undetected, especially in heterozygous females. Recently, MLPA has been broadly applied to gene mutation screening

to detect exon deletions and duplications. Different deletions were detected using MLPA assay on 25 patients affected by severe haemophilia A, resulted mutation negative by sequencing analysis. Traditional PCR failed to amplify one or more exons in some of these patients and we decided to use the MLPA test in order to confirm the conjectured deletions: 7 deletions were revealed in haemophiliacs patients and we identified the carrier status in 2 female.

F8 mutational screening could be improved by adding MLPA to sequence analysis and MLPA is a helpful tool in order to define the status of carriers in female relatives of haemophiliacs with deletions of one or more exons of F8 gene. It is to underline the importance of performing a molecular analysis in females suspected haemophiliacs carriers: the main obstacle to their counselling is the impossibility to demonstrate a heterozygous status because of the amplification of the exon/s that is/are present on the normal X chromosome. So, the only way to make a definitive diagnosis is to detect the mutation in F8 gene and MLPA provides an important tool for the detection of its complete mutational spectrum.

#### P12.080

#### Genotyping of Coagulation Factor IX gene in Hemophilia B Patients of Esfahan Province

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Hemophilia B, Christmas disease, is an X-linked bleeding disorder caused by the functional deficiency of blood coagulation factor IX. The disease is due to heterogeneous mutations in the factor IX gene (*F9*), located at Xq27.1. It spans about 34 kilobases (kb) of genomic DNA. The aim of this study was molecular analysis and genotype-phenotype correlation of hemophilia B patients in Isfahan province, Iran. After obtaining informed consent, genomic DNA was extracted from the peripheral blood of 37 patients referred from Isfahan hemophilia center, by standard methods. PCR amplification, SSCP and CSGE techniques were performed for scanning of the all functional-important regions of the *F9* gene. DNA sequencing were performed for those with different migration patterns in SSCP or CSGE by chain termination method. In addition, haplotype were constructed using four the Ddel, Taql, Hhal and Mnll restriction fragment length polymorphisms (RFLPs) markers. The sequencing results showed 70.3% missense mutation, 18.9% nonsense mutation, 8.1% deletion, 2.7% insertion. In this study, of the 19 hemophilia B patients of the Kashan, all of them were represented substitution (G6472A) that could represent a founder effect. Five novel mutations which have not been reported in hemophilia B mutation database, were also found. The information obtained from this study could be used to diagnose potential female carriers in families with hemophilia B patients and prenatal diagnosis.

#### P12.082

#### Molecular screening of 980 cases of suspected hereditary optic neuropathy with a report on 77 novel OPA1 mutations

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We report the results of molecular screening in 980 patients carried out as part of their work-up for suspected hereditary optic neuropathies. Among these patients, 588 (60%) had a family history of hereditary optic atrophy whereas the remaining 392 (40%) patients had no obvious family history of the disease. All the patients were investigated for Leber's hereditary optic neuropathy (LHON) and autosomal dominant optic atrophy (ADOA), by searching for the ten primary LHON-causing mtDNA mutations and examining the entire coding sequences of the OPA1 and OPA3 genes, the two genes currently identified in ADOA. Molecular defects were identified in 440 patients (45% of screened patients). Among these, 295 patients (67%) had an OPA1 mutation, 131 patients (30%) had an mtDNA mutation, and 14 patients (3%), belonging to three unrelated families, had an OPA3 mutation. Interestingly, OPA1 mutations were found in 157 (40%) of the 392 apparently sporadic cases of optic atrophy. The eOPA1 locus-specific database now contains a total of 204 OPA1 mutations, including 77 novel OPA1 mutations reported here. The statistical analysis of this large set of mutations has led us to propose a diagnostic strategy that should help with the molecular work-up of optic neuropathies. Our results highlight the importance of investigating LHON-causing mtDNA mutations as well as OPA1 and OPA3 mutations in cases of suspected hereditary optic neuropathy, even in absence of a family history of the disease.

#### P12.083

#### Synergistic effect of spastin gene mutations in a Hungarian patient with hereditary spastic paraparesis (HSP)

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Hereditary spastic paraparesis (HSP) is a group of genetically heterogeneous disorders characterized by progressive spasticity of the lower limbs. Mutations in the *SPG4* gene, which encodes spastin protein, are responsible for up to 45% of autosomal dominant cases.

Here we present a Hungarian family, in which the 31 year old proband has pronounced progressive spastic paraparesis, increased deep tendon reflexes and pyramidal signs in the lower limbs. She suffers from urinary incontinence. The proband was born with club feet. Her 58 year old mother's symptoms started at age 48 with mild walking problems. She has mild spastic paraparesis, increased deep tendon reflexes and pyramidal signs in the lower limbs. DNA analysis by direct sequencing of all exons in *SPG1*, *SPA2*, *SPG3a* and *SPG4* genes revealed the following: the proband is compound heterozygous for the sequence variants C131T (S44L) and C1684T (R562Term) in the spastin (*SPG4*) gene. In her mother only the C1684T (R562Term) mutation was detected. The C1684T substitution has been previously described as a pathogenic mutation. Although the S44L amino acid change has been described in the literature as a pathogenic mutation more current data suggest this change is a harmless polymorphism (rs28939368) if it occurs alone. In the presence of a disease causing mutation it may act as a modifier, leading to an earlier age of onset.

We can conclude that in the proband the coexistence of S44L and R562Term resulted in the severity clinical symptoms and early age of onset.

#### P12.084

#### Frequency of *SPG42* in Autosomal-Dominant Spastic Paraparesis (AD-HSP)

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**Background:** Hereditary spastic paraparesis (HSP) represents a neurodegenerative disorder resulting in progressive spasticity of the lower limbs. The most frequent causes of autosomal dominant HSP (AD-HSP) are mutations in the *SPAST*-gene (SPG4 locus). However, roughly 60% of the patients remain SPG4 mutation-negative despite a family history compatible with autosomal dominant inheritance. Mutations in the gene for acetyl-CoA transporter (SLC33A1) have recently been reported to cause autosomal dominant hereditary spastic paraparesis (HSP) type SPG42.

**Objective:** We wanted to determine the relative frequency of SPG42/ SLC33A1 mutations in index patients of AD-HSP families for whom SPG4 mutations had been excluded.

**Methods:** We screened a large cohort of 263 SPG4 mutation-negative patients for variations in SPG42/SLC33A1 by high resolution melting (HRM) combined with direct sequencing.

**Results:** Currently, aberrant melting curves were identified in 32 cases (12%). Subject to future investigation will be, for each resulting aberrant HRM curve the direct sequencing of the corresponding sample due to assess a variation. Further a multiplex ligation dependent probe amplification assay (MLPA) targeting SPG42 will investigate copy number variations.

#### P12.085

#### Only rarely is thin corpus callosum (TCC) present in SPG11 - clinical heterogeneity and genetic data in a large international cohort

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Spatacsin mutations cause an autosomal - recessive (AR) form of spastic paraparesis, SPG type 11 (SPG11). Typically SPG11 is been diagnosed in cases with slight ataxia, thin corpus callosum (TCC), mental impairment, pyramidal signs, increased deep tendon reflexes and only rarely neuropathy. In a cohort of 251 patients with AR-HSP we analysed SPG11 gene by sequencing the gene including the exon-intron boundaries and deletion screening by MLPA. We have been able to detect 88 mutated alleles in 44 patients from 37 non-related families. Patients are from Canada (N=11), Arabian countries (N=4), Scandinavia (N=3), East-Europe (N=9), Latin-America (N=2), 8 from Singapore, UK, Denmark, Spain, Germany, Austria and Croatia, resp. We found 64 different mutations (37 are not described): splice mutations 22/88 (25%), small deletions/insertions 38/88 (43%), large deletions (exon 11, 24, 27, 29, 31, 32, 34) 10/88 (11%), missense/nonsense mutations 18/88 (20%). For 38 patients we got clinical data including MRI results (35 cases). Age at onset ranged from 8 to 38 years with a mean of 22.0+/-9.6. Onset was characterized by gait disorders (21/38, 55%), polyneuropathy (7/38, 18%), dementia, dystonia, tremor and dysarthria (each in three patients). Interestingly only in 15/35 (42%) a TCC has been demonstrable. This low frequency of TCC is in contrast to other reports and raises the question of the specificity of TCC for SPG11. Mutations within the exons 24 - 34 are correlated with a more severe phenotype. Our data demonstrate a great phenotypic variability and a high percentage of large deletions within the SPG11 gene

**P12.086****Genetic heterogeneity of hereditary neuropathy with liability to pressure palsies at the Russian patients**

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Hereditary neuropathy with liability to pressure palsies (HNPP) is an autosomal dominant painless peripheral neuropathy characterized by episodes of repeated focal pressure neuropathies at sites of entrapment/compression, with a considerable variability in the clinical course.

Forty patients and twenty one healthy members from 26 unrelated families with a clinical history and examination suggestive of HNPP were observed. In 9 families HNPP were due to the common 1.5 Mb deletion. This mutation was detected on the basis of a PCR result that demonstrated loss of heterozygosity for each of four polymorphic markers in 17p11.2-12 region. Multiplex Ligation-dependent Probe Analysis (MLPA) was carried out for all patients and family members using a probes were designed to evaluate all PMP22 coding exons and five control genes: TBP, SIRT3, USP3, B2M and EEF. Disease-cause deletion was found at the three more unrelated families. At the three cases deletion de novo was the reason of disease and in the others families mutation persistence at several generations. At one patient the disease-cause duplication of 17p11.2-12 region were detect.

In the absence of the deletion/duplication direct sequence analysis of the all PMP22 coding exons 2-5, including their intron/exon boundaries was undertaken. One c.353C>T (Thr118Met) the previously described mutation in a heterozygotic condition and one new mutation c.75\_78+2delCAGCgt were detected in two families.

Thereby exciting cause was found in total at 15 from 26 unrelated families. In summary, we were able to detect genetic lesions in over 60% of HNPP patients from Russia.

**P12.087****Huntington disease pathogenesis: molecular biomarker/s in human peripheral blood and clonally striata-derived rat cells**

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Huntington disease (HD) is an inherited neurodegenerative disease characterized by chorea and psychiatric disturbance. It is caused by the expansion of CAG repeats in the first exon of the gene encoding huntingtin (Htt). Normal people have a polymorphic repeat, up to 36 CAG, encoding for glutamine (Gln). The mutant protein, with a longer poly-Q tract, becomes harmful to cells, particularly to cortical and striatal neurons. Mutant Htt affects many cellular processes, calcium homeostasis and mitochondrial dysfunction between others, still investigated to unveil their cause and effect relationship. An important tool for HD management could be the identification of molecular markers related to disease progression, in symptomatic and pre-symptomatic people, useful to draw early therapeutic treatments. By using quantitative RT-PCR we investigate the expression level of genes involved in calcium homeostasis in the peripheral blood of HD subjects and age- and gender- matched healthy controls. The genes selected for this study were: a purinergic receptor, P2RY5, and three calcium pumps, SERCA2, SERCA3 and PMCA1. The results we obtained demonstrate that, with respect to the controls HD patients display: 1) similar levels of SERCA3 and PMCA1 mRNAs. 2) a significant reduction of P2RY5 mRNA level, in an age-related progression. 3) a significant reduction of SERCA2 mRNA level at all ages. Furthermore, we propose SERCA2 reduction as an early event in HD pathogenesis, since this pump is down-regulated at the protein level in clonally striata-derived rat cells expressing a doxycycline-inducible mutant Htt.

**P12.088****Huntington disease pathogenesis: molecular biomarker/s in human peripheral blood and clonally striata-derived rat cells**

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Huntington disease (HD) is an inherited neurodegenerative disease characterized by chorea and psychiatric disturbance. It is caused by

the expansion of CAG repeats in the first exon of the gene encoding huntingtin (Htt). Normal people have a polymorphic repeat, up to 36 CAG, encoding for glutamine (Gln). The mutant protein, with a longer poly-Q tract, becomes harmful to cells, particularly to cortical and striatal neurons. Mutant Htt affects many cellular processes, calcium homeostasis and mitochondrial dysfunction between others, still investigated to unveil their cause and effect relationship. An important tool for HD management could be the identification of molecular markers related to disease progression, in symptomatic and pre-symptomatic people, useful to draw early therapeutic treatments. By using quantitative RT-PCR we investigate the expression level of genes involved in calcium homeostasis in the peripheral blood of HD subjects and age- and gender- matched healthy controls. The genes selected for this study were: a purinergic receptor, P2RY5, and three calcium pumps, SERCA2, SERCA3 and PMCA1. The results we obtained demonstrate that, with respect to the controls HD patients display: 1) similar levels of SERCA3 and PMCA1 mRNAs. 2) a significant reduction of P2RY5 mRNA level, in an age-related progression. 3) a significant reduction of SERCA2 mRNA level at all ages. Furthermore, we propose SERCA2 reduction as an early event in HD pathogenesis, since this pump is down-regulated at the protein level in clonally striata-derived rat cells expressing a doxycycline-inducible mutant Htt.

**P12.089****Exclusion of trinucleotide repeat expansions in JPH3 gene causing disease in Italian patients with Huntington-like phenotype**

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Huntington disease-like 2 (HDL2) is a rare autosomal dominant disorder of the nervous system, apparently indistinguishable from Huntington disease (HD). HDL2 belongs to a group of HDL disorders, also including Huntington disease-like 1 (HDL1), an autosomal dominant disease caused by an extra octapeptide repeat in the prion protein gene (PRNP), on chromosome 20p12; and Huntington disease-like 3 (HDL3), an autosomal recessive disease, mapped to 4p15.3. Furthermore, dentatorubral-pallidoluysian atrophy (DRPLA) and spinocerebellar atrophy type 17 (SCA17), may also have overlapping symptoms with HD.

HDL2 is caused by the expansion above 40 CAG repeats, in a variably spliced exon of the junctophilin-3 gene (JPH3), on chromosome 16q24.3. The pathogenic mechanism underlying HDL2 remains unknown. Different JPH3 transcripts caused by alternative splicing and different polyadenylation sites have been described.

To date, the expansion in JPH3 gene has been found only in patients with African ancestry. A very recent paper reports the first HDL2 case with an apparent European ancestry. In order to find a new founder effect on Caucasian population, we performed a mutational analysis in JPH3 gene in 132 Italian patients negative for HD disease. All patients tested were negative for the expansion on JPH3 according to the available data on Polish, Yugoslavian and Portuguese populations.

Further investigations for HDL1 and HDL3 forms, as well as DRPLA and SCA17, will be performed on our patients. However, the diagnosis of HDL2 should always be considered, regardless of the apparent origin of the family, particularly in populations of mixed ancestry.

**P12.090****PCR of (CAG)n repeats in Bulgarian Huntington Horea patients**

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Huntington disease (HD) is an autosomal dominant neurodegenerative disorder that gives rise to progressive, selective (localized) neural cell death associated with choreic movements, rigidity and dementia frequently associated with seizures. The disease is associated with increases in the length of a CAG triplet repeat in IT15 gene called 'huntingtin' located on chromosome 4p16.3.

The normal size of the CAG triplet of the HD is  $\leq 35$  repeats, the pathological number of the CAG triplet is larger than 36 repeats with different penetrance (36-39 repeats - <90% penetrance; 40-41 repeats - 90-99% penetrance; 42 repeats or more - 100% penetrance).

We developed a new PCR protocol for precise calculation of (CAG)n repeats using high betaine concentration. The amplification products were separated by capillary electrophoresis on ABI 310 genetic analyzer (Applied Biosystems). Using this protocol the CAG triple repeats could be precisely measured by size. We analyzed 12 individuals from 5 unrelated Huntington families. Seven individuals were found to be affected. The expanded fragments were measured to have 39, 41, 42, 43, 46 and 49 CAG repeats respectively. In all families the CAG triplet expands further in the next generation. The proposed diagnostic method is very fast, cheap and easy to perform, which makes it very useful in routine laboratory work. It can totally substitute the utilized so far Southern blot analysis

#### P12.091

##### Structural analysis of the huntingtin protein

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Huntington's Disease (HD) is an autosomal dominant neurodegenerative disorder. The mutation underlying HD is the expansion of the polymorphic CAG tract in the first exon. Despite intensive research, molecular pathology of HD has not been fully understood. In this study, threading method is used to model the huntingtin protein. By using the VMD programme, various glutamine expansion mutations are created and changes in the protein structures are analyzed. The most convenient five models of huntingtin indicated the dominance of  $\alpha$ -helices. Where  $\alpha$ -helices are less common, long turns, coils and  $\beta$ -sheets are recognized. In addition to that, mutant proteins with expanded glutamines are created *in silico* and their structures are compared to the most probable huntingtin structure, 1WA5\_B. According to the results, mutant proteins with 33-38Q did not show any significant change, however 39 or more glutamines caused conformational changes like lengthened and increased turns and coils and shortened  $\alpha$ -helices. In this situation, protein may gain non-covalent interactions within itself or with other proteins and aggregate in the form of twisted  $\beta$ -sheets. These changes may render the protein more prone to proteolysis. In this way, toxic protein fragments carrying the glutamine tract are favored. On the other hand, usual proteolysis may not take place, which may cause toxicity by increasing the half life of the protein. Also, conformational change may lead to misfolding and aggregation of the proteolysis-resistant protein in the cell. Thus, conformational changes in the mutant huntingtin give some clues about the neurodegenerative pathways in HD.

#### P12.092

##### Somatic instability of CAG repeats in Huntington's Disease: role of oxidative DNA damage and Base Excision Repair

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Huntington's disease (HD) is a neurodegenerative disorder, caused by CAG repeats extension encoding a toxic polyglutamine expansion in the huntingtin protein. Instability of the HD mutation leads to age-dependent and tissue-specific somatic expansion. Interestingly, the brain region preferentially degenerating, the striatum, is highly unstable, while the disease-spared cerebellum is rather stable. Recently, the glycosylase *Ogg1*, which initiates Base Excision Repair (BER), was shown to promote somatic instability, suggesting that oxidative DNA damage and BER drive this process.

We show that oxidative DNA damage is specifically increased at the CAG-expanded locus in R6/1 HD mice. Surprisingly, levels of oxidative lesions are similar in striatum and cerebellum, and in young and aged tissues. Thus, accumulation of oxidative lesions at the CAG repeats is neither tissue- nor age-dependent, indicating that oxidative DNA damage at the CAG-expanded locus is necessary but not sufficient to trigger somatic instability. Protein levels and activities of major BER players were analyzed in the striatum and in the cerebellum of R6/1 mice. We show in particular that FEN1, the flappy endonuclease involved in Long-Patch BER (LP-BER) subpathway, is much less active in the striatum than in the cerebellum of R6/1 mice. Using chromatin immunoprecipitation, we finally show that POL $\beta$  is specifically enriched at the CAG-expanded locus in the striatum of aged R6/1 mice, but not in their cerebellum. This supports that LP-BER triggers somatic CAG expansion in the aging striatum, the inappropriate stoichiometry

of POL $\beta$  and FEN1 resulting in poor coordination of polymerase strand displacement and flappy endonuclease activities.

#### P12.093

##### Sporadic In Utero Generalized Edema Caused by Mutations in the Lymphangiogenic Genes VEGFR3 and FOXC2

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Hydrops fetalis is a serious fetal condition with high mortality rate. It is mostly sporadic; the etiology is unknown in about 25% of cases. In this study, we investigated the genetic causes of idiopathic sporadic prenatal generalized edema. In a series of 12 patients, in whom in utero generalized skin edema or hydrops fetalis had been diagnosed, three lymphangiogenic genes, VEGFR3, FOXC2 and SOX18, were screened. In three of the patients, we identified a mutation: two in VEGFR3 and one in FOXC2. Two of the mutations were *de novo* and one was either *de novo* or non-penetrant inherited. In these patients, the generalized edema resorbed spontaneously, either in utero or after birth. In the two individuals with a VEGFR3 mutation, edema remained limited to lower limbs. Therefore, mutations in the VEGFR3 and FOXC2 genes account for a subset of patients with unexplained in utero generalized subcutaneous edema and hydrops fetalis without family history of lymphedema. These data expand our understanding of the etiology of these phenotypes and suggest that lymphangiogenic genes should be screened for mutations in sporadic patients diagnosed with fetal edema.

#### P12.094

##### Hyperferritinémia and mutations in the promoter of HAMP

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Hepcidin (gene HAMP, 19q13) is a protein which is supposed to regulate iron uptake or recycling through binding to the iron export protein: the ferroportin (gene SLC 40 A1, 2q32). It is secreted by the liver and considered as a key iron-regulatory hormone linking the liver's "iron sensor" to bowel absorption. High iron storage induce the expression of hepcidin and thus the decrease of absorption. The Molecular defects of its gene are associated with a class of hyperferritinemia called hemochromatosis type 2b (OMIM : 606464). We have screened the HAMP gene in patients displaying abnormal iron parameters not linked to a mutation in HFE or SCL40 A1 genes. We found two new mutations in the promoter: c. -153 C>G and c. -188 C>T. The first variant affects a binding site for BMP which was described to control hepcidin promoter activation. Functional studies of other mutations located in this binding site confirmed its' functional importance (G. Legac and J. Rochette, personal communication). The second one was found several time in patients originated from Africa and screened for iron metabolism disorders. As it was recently published [J. Mol Med, (2008) 86:531-540], the proximal part of the promoter plays a crucial role in the regulation of hepcidin expression. These observations could represent natural variants of hepcidin expression allowing to understand the regulation pathway of this gene and consequences of its perturbation. Functional study of the second mutation is ongoing

#### P12.095

##### Probing the oligogenic nature of idiopathic chronic pancreatitis: first analysis of five susceptibility genes in a large cohort of young patients

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Introduction: Chronic pancreatitis (CP) is an inflammatory disease of the pancreas. In developed countries, alcohol explains the majority of the CP cases, followed by "idiopathic" causes which account for about 20% of the cases. Mutations in five genes, the cationic trypsinogen (PRSS1) gene, the pancreatic secretory trypsin inhibitor (SPINK1) gene, the cystic fibrosis transmembrane conductance regulator (CFTR) gene, the calcium sensing receptor (CASR) gene and the chymotrypsinogen C (CTRC) gene, have been found to be associated with CP. In order to determine the relative contribution of these genes in idiopathic CP (ICP), we recruited 239 patients who have developed the disease before 20 years.

Methods: Coding regions of each gene were analyzed by either denaturing high-performance liquid chromatography, high-resolution melting or direct sequencing. Quantitative fluorescent multiplex PCR was performed to screen genomic rearrangements.

Results: Some 50% of the ICP patients carried at least one mutation in one of these genes. First, about 30% of the patients carried at least one mutation in the *CFTR* gene including the IVS8-T5 variant, and 10% of the patients were compound heterozygotes (mild/mild or severe/mild). Moreover, the p.N34S polymorphism of *SPINK1* was found in about 15% of patients and 14 patients carried one mutation in the *CTRC* gene. "Gain of function" mutations in the *PRSS1* gene (11/239) and *CASR* mutations (4/239) were rarely found. Finally, 27 patients were trans-heterozygotes.

Conclusion: These results demonstrated that about 50% of the patients labeled as ICP had, in fact, a genetic defect, and revealed the complex nature of ICP.

#### P12.096

#### Mutation scanning in the *IKBKG* gene in Incontinentia Pigmenti, methods and results

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Incontinentia pigmenti (IP) is a rare neurocutaneous X-dominantly inherited disorder, prevalence approximately 1/50,000. Besides skin and neurological abnormalities, there is ophthalmologic and dental involvement. The skin abnormalities appear in three stages, starting in the neonatal period. After childhood hypopigmented lines are left. Skin abnormalities follow the lines of Blaschko. IP is observed almost exclusively in girls, affected boys die in utero. Female patients show extremely skewed X-inactivation in DNA isolated from blood. Rare surviving male patients are mostly mosaics or have an XXY karyotype. IP is caused by mutations in the *IKBKG* gene (kinase of inhibitor of kappa light polypeptide gene enhancer in B cells gamma, also called *NEMO*), on Xq28. Mutation scanning of *IKBKG* is complicated by the presence of a flanking pseudogene, identical to the functional *IKBKG* gene only lacking exons 1 and 2. In most IP patients the causative mutation in *IKBKG* is a deletion of exons 4-10. This mutation can be identified by a specific PCR reaction. If no deletion is detected all exons are sequenced following specific amplification of the functional *IKBKG* gene by long-range PCR. We screened 79 index cases, 9 were males. We identified the classical deletion in 37 female patients. In addition, we identified 11 different mutations in 11 female patients. In only 9 patients with extremely skewed X-inactivation no mutation was identified. In none of the male cases a mutation was found. In 30% of cases the deletion appeared *de novo*. All mutations other than the classical deletion introduce premature stopcodons.

#### P12.097

#### Novel point mutations in the senataxin gene of three patient with ataxia and oculomotor apraxia type 2 (AOA2)

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AOA2 characterized by progressive cerebellar atrophy, axonal neuropathy and increased AFP. It is caused by mutations in the SETX gene. To date more than 35 mutations in 65 patients have been described in the literature. Here we describe 3 AOA2 patients harboring new senataxin mutations. The 22 year old woman's (P1) symptoms

started at age 15 with unsteady gait. Presently she has moderate gait ataxia which worsens episodically and in association with vertigo. Examination revealed moderate truncal and limb ataxia. The 23 year old male's (P2) symptoms started at age 12 with imbalance, 2 years later dysarthria and episodic diplopia occurred. Examination revealed oculomotor apraxia, nystagmus, dysarthria, distal atrophy and spasticity of the legs, increased reflexes, pyramidal signs, truncal and limb ataxia. The 54 year old male (P3) has a progressive imbalance since the age of 40. Now he has oculomotor apraxia, truncal and limb ataxia, and parkinsonian symptoms. All patients had increased AFP and cerebellar atrophy. Sequence analysis of the SETX gene in all patients revealed novel mutations: P1 had homozygous R1606X mutation resulting in premature termination of gene translation before the helicase domain. P2 was compound heterozygous for the L2155W and R2444C mutations in the helicase domain. P3 had homozygous T1854A mutation localized close to the helicase domain. In conclusion: patients with mutations resulting in dysfunction of helicase domain have earlier age of onset and more severe clinical symptoms. This could be explained by the role of this domain in DNA repair, replication and RNA splicing.

#### P12.098

#### Allelic heterogeneity of *TMPRSS6* mutations

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Matriptase-2 is a transmembrane serine protease encoded by the *TM-PRSS6* gene that negatively regulates hepcidin expression. *TM-PRSS6* mutations have been reported in several patients with iron deficiency anemia refractory to oral iron therapy (IRIDA), an autosomal recessive disease characterised by low serum iron, low transferrin saturation and inappropriate high level of plasma hepcidin. Here we report 8 novel cases from 6 families with *TM-PRSS6* mutations. Four patients were compound heterozygotes for 2 missense mutations targeting different subdomains (families I,II,III). Two brothers (family IV) were homozygous for a frameshift deletion. Finally, in two patients (cases 7,8) the mutation was found in only one allele. A large range of serum hepcidin concentration was found in different patients. All patients but two brothers (family II) were not sensitive to oral iron supplementation and had received IV iron which resulted in an increase in the serum ferritin and almost complete correction of the anemia. After a course of IV iron, the response was usually sustained, with hemoglobin levels reaching near normal values for several months. This observation supports the idea that in the presence of abnormally elevated hepcidin levels, a part of infused iron is sequestered in macrophages then is subsequently slowly released and utilized by the erythropoietic cells. Our results suggest that IRIDA is a relatively benign genetic disease provided appropriate therapy is given to the patients.

Family Case	<i>TM-PRSS6</i> genotype in affected subjects\$	Matriptase-2 protein domain modified/ aa change	age at diagnosis	Hb at diagnosis (g/dl)	Current age	serum hepcidin (ng/ml)*\$	ferritin (μg/dl)\$	Hb (g/dl)\$
I 1	[c.1561 G>A]H[c.1564 G>A]	LDLRA2/LDLRA2 [p.D521N]+[p.E522K]	1y	10	11y	443	347	11.9
II 2, 3	[c.1253A>G]H[c.704 T>C]	CUB1/CUB2 [p.L235P]+[p.Y418C]a	6y	7.7	11y	62	90	12
III 4	[c.2283 C>G(+) c.340 G>A]	SEA/SP [p.E114K (+)p.T765A]	7m	9	8y	81	115	12
IV 5, 6	[c.1813 delG]+[c.1813 delG]	[p.A605PfsX8]+[p.A605PfsX8]p	4y	10.2	7y	ND	20	10.8
V 7	[c.1369+4 A>T]+[?]	[splicing mutation]+[?]	15y	7.6	28	4288	230	12
VI 8	[c.335 G>T]+[?]	SEA/ ? [p.R112L]+[?]	11y	7.4	17	ND	66	12
						87#	28#	9.2#
						40y	ND	12

\$ cDNA sequence reference AY055384

\* normal range : 28-244 ng/ml (unpublished data, Ganz et al)

§ current values for patients under iron therapy (oral or IV) excepted for patient 7, indicated by #. Hb of patient 7 reached 11.5 in 2001 after IV iron therapy.

□ patient also heterozygote for the p.R446W

£ donor splice site of intron 11 is predicted to be suppressed

abbreviations : IRIDA : iron-refractory iron deficiency anemia, LDLRA:low density lipoprotein receptor class A domain, CUB : complement factor C1rC1s, urchin embryonic growth factor and bone morphogenetic protein, SEA : sea urchin sperm protein, enteropeptidase, agrin, SP: serine protease, y year, m month

#### P12.099

#### The first report of IVSI-II(T>C) from Iran in three $\beta$ -Thalassemia carrier

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$\beta$ -Thalassemia is the most frequent single gene disorder in Iran. The cause of the disease is defect in the synthesis of  $\beta$ - globin chain and has been reported from different parts of the world. Ordinarily, in any population there are specific mutations. The type of  $\beta$ -thalassemia mutation has influence on the  $\beta$ - globin chain synthesis represented by  $\beta^0$ ,  $\beta^+$  and  $\beta^{++}$  thalassemia.  $\beta$  -Thalassemia is very prevalent in northern provinces of Iran and the most regional a common mutation is IV-SII-I(G>A). Some of the  $\beta$ -Thalassemia carriers referred to our clinic for mutation detection remained uncharacterized after ARMS analysis for known mutations. Among These individuals, three carriers were identified by direct sequencing of the PCR-amplified product, which had T>C substitution HBB.C.92+2 T>C (IVSI-II). Here we report a mutation in  $\beta$ -globin gene found in three individual carriers of  $\beta$ -Thalassemia referred to our lab. After obtaining written informed consent, genomic DNA was extracted from peripheral leukocytes by salting out method. ARMS-PCR was exploited for detecting common mutations and DNA sequencing was performed for finding unknown mutations in  $\beta$ -globin gene. Among carreiers of  $\beta$  -Thalassemia referred to us three had the above mutation. To our best knowledge this is the first report of this mutation from Iran. The indices (MCV and MCH) and HbA<sub>2</sub> of these carriers were like reports in globin gene server.

#### P12.100

#### Mutations spectrum of juvenile polyposis syndrome in Poland

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Juvenile polyposis (JP) is a genetically determined predisposition to occurrence of multiple juvenile polyps in gastrointestinal tract and associated malformations such as: porphyria, psoriasis, mental retardation, congenital heart disease, cleft lip and palate, epilepsy, hereditary haemorrhagic telangiectasia, digital clubbing in children. Hypertrophic pulmonary osteoarthropathy and malrotation of the gut also could be observed. Prevalence of JP syndrome is 1 per 100000. The JP syndrome is inherited in an autosomal dominant manner and the risk of malignancy in JPS patient is increased and could be ranged more then 60% in alimentary tract. We performed mutation analysis in DNA isolated from 17 Polish patients with JPS. The entire coding sequence of the BMPR1A and SMAD4 genes were studied. We used methods SSCP and HA analysis for mutation screening. The DNA fragment presenting different migration patterns on the polyacrylamide gels were sequenced by direct PCR product sequencing using automated DNA sequencer according to manufacturer's instruction. In result of molecular investigations we observed five mutations and ten exonic polymorphism and intronic variations. Moreover, using the Multiplex Ligation-dependent Probe Amplification (MLPA) - method with kit P158-A1 (MCR Holland) we were able to detected five additional large mutations. Detected the genomic deletions have size ranging from one exon to two whole genes. In our study in one case we observed deletion both PTEN and BMPR1A genes.

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#### P12.101

#### Karak syndrome in two Saudi Arabian families with linkage to PLA2G6 locus

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A hetererogeneous group of severe neurological disorders like Aceruloplasminaemia, Neuroferritinopathy, Hallervorden-Spatz syndrome, HARP syndrome, and Fridreich ataxia involve excess brain iron accumulation. The "eye of the tiger" sign is a common neuroradiological finding in neurodegeneration with iron brain accumulation type 1 and 2 (NBIA1, 2), infantile neuroaxonal dystrophy (INAD) and Karak syndrome (KS). Mutations in PANK2 and PLA2G6 genes were implicated in NBIA, INAD and KS disorders. We describe 2 consanguineous Saudi families with Karak syndrome. These consisted of 4 affected individuals (1 male and 3 females, aged 5 - 24 years). Onset ranged between 1 and 7 years with progressive cerebellar ataxia and spasticity associated, later, with extrapyramidal signs, intellectual decline and axonal form of Charcot-Marie- Tooth disease (CMT2). Ambulation was lost between 4 1/2 and 15 years. One male patient died at 24 years. Ophthalmic evaluations revealed abnormal vertical saccades and pursuit. Brain MRI showed iron deposition in the putamen in all patients. These 2 families were genotyped for PANK2 and PLA2G6 loci using 10 microsatellite markers. PANK2 locus was excluded while assignment of the families to the PLA2G6 locus was established by homozygosity mapping. Direct sequencing of the PLA2G6 gene is in progress as well as the genotyping of 3 other consanguineous families with the same phenotype and belonging to same country. The results of these investigations will be shown during the meeting.

#### P12.102

#### Molecular genetic analysis of families with Keratosis Follicularis Spinularis Decalvans in a refined KFSD locus

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KFSD (Keratosis Follicularis Spinularis Decalvans, OMIM 308800) is a rare genetic disorder affecting both skin and eyes. It is characterized by follicular hyperkeratosis of the skin developing into patchy scarring alopecia and loss of the follicles of the hair, eyelashes, and eyebrows. Associated eye symptoms include photophobia in childhood and/or blepharitis and corneal dystrophy. Due to the clinical heterogeneity of KFSD the definitive diagnosis is often challenging. KFSD closely resembles other disorders where abnormal keratinization is involved such as keratosis pilaris atrophicans faciei (KPAF), atrophoderma vermiculatum (AV) and ichthyosis follicularis with atrichia and photophobia (IFAP). The question remains whether these syndromes are simply variations of the same entity or truly independent.

Although an X-linked pattern of inheritance has been confirmed in two different families, the existence of a rare autosomal dominant variant has been postulated. Linkage and recombination analysis in a large Dutch family identified two key recombinants mapping the KFSD locus to Xp22.11-p22.13. Towards identification of the causative gene, we studied the large Dutch family and together with some other families with a clinical diagnosis of KFSD using new molecular tools. 1M SNP arrays were used to refine the locus to a 2.9 Mb region and to exclude the involvement of large deletions and duplications. At present, the 14 genes in the candidate gene interval are screened for possible pathogenic variants. Finally, whole genome gene expression profiling is currently used to study differences between patient and control fibroblasts.

## P12.103

## Novel RDH12 and RPE65 gene variants associated Congenital Amaurosis with Leber

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Leber Congenital Amaurosis (LCA) is a severe retinal dystrophy involving both cone and rod systems and causing severe visual impairment.

LCA is inherited in an autosomal recessive manner. Actually 11 genes have been identified to be involved in the pathogenesis of the disease: AIPL1, CRB1, CRX, LRAT, GUCY2D, IMPDH1, TULP1, RDH12, RPE65, RPGRIP1, CEP290.

Even if these genes code for proteins playing different biological role, there is not a wide-range of phenotypes related to different mutations, and the clinical features related to different mutations in different genes seems to be similar.

In this study 3 patients of 3 different families were examined for RDH12, RPE65 e GUCY2D genes, by direct sequencing.

The first patient showed one novel RDH12 missense variant (p.Val233Glu) in homozygous state, and one previously described missense mutation (p.Pro701Ser) in GUCY2D gene, in heterozygous state.

The second patient showed one novel RDH12 stop variant (p.Trp304Stop), in homozygous state. The same mutation was identified in both clinically healthy heterozygote parents of this patient.

The third patient showed two RPE65 mutations in heterozygous state: the novel variant c.440\_441delCA, and the described mutation p.Arg91Trp.

Large-scale molecular screening of LCA-associated genes will probably permit a better understanding of the physiopathological consequences of the different gene mutations with more reliable prognostic evaluations in clinical practice.

## P12.104

## A new splicing mutation in HPRT1 gene in a Romany boy - a genotype-phenotype reference

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Lesch-Nyhan syndrome is an X-linked disorder of purine metabolism caused by Hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency. Mild forms are termed X-linked hyperuricaemia or Kelly-Segmiller syndrome, clinically presenting with gout, eventually also mild neurological symptoms. Severe HPRT deficiency additionally leads to nephrolithiasis with chronic renal failure and serious neurological impairment - psychomotor retardation, hypotonia, automutilations. The Romany patient described here clinically presented with acute renal failure already in newborn age. His present status (age 3 years) represents severe delay - no sitting, no speech, hypotonia, dyskinesia. Self-biting started in his 2nd year of life. Renal functions are reduced, ultrasound shows kidney calcifications.

Analyzing a HPRT1 gene of the patient, we have identified a novel splicing mutation c.27+2T>C in intron 1 (IVS 1+2T>C). The influence of mutation's impact to mRNA splicing was evaluated by cDNA analysis. Sequencing of cDNA containing exons 1,2 and a part of 3, proved defective splicing of mRNA. Mutation c.27+2T>C abolishes the natural donor splice site and an alternative splice site within intron 1 is used (r.27\_28ins49). The protein translated from the mutated RNA is predicted to contain only 26 amino acid residues. However, according to our results, the majority of mutated mRNA undergoes nonsense-mediated mRNA decay and the defective protein is not synthesized.

The severe mutation described here occurred de novo in the patient and led to full-blown Lesch-Nyhan syndrome. Our results in accordance with published data point to a good genotype-phenotype correlation in patients with HPRT deficiency.

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## P12.105

C-terminal deletions of y<sup>+</sup>LAT-1 do not affect the dimerization of y<sup>+</sup>LAT-1/4F2hc transporter but can cause a targeting defect.

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Lysinuric protein intolerance (LPI) is an autosomal recessive disorder of cationic amino acid transport at the basolateral plasma membrane caused by mutations in the SLC7A7 gene encoding y<sup>+</sup>LAT-1 protein. The active amino acid transporter consists of the light subunit y<sup>+</sup>LAT-1, which determines the substrate specificity as the heavy subunit 4F2hc guides the complex to the membrane. 4F2hc also regulates other cellular functions including cell activation, proliferation, survival and apoptosis.

We reported previously that y<sup>+</sup>LAT-1 protein with LPI missense mutation is carried to the plasma membrane, while frameshift mutants are cytoplasmic. Also, truncated y<sup>+</sup>LAT-1s lacking part of C-terminal tail are correctly localized while larger deletions remain subcellular. However, the y<sup>+</sup>LAT-1-4F2hc interaction occurs regardless of the targeting mutations. In the current study, we use C-terminal deletions of y<sup>+</sup>LAT-1 to study their dimerization using flow cytometry FRET and the effects of their expression on the cells.

Our results indicate that the interaction of y<sup>+</sup>LAT-1 and 4F2hc within the cell is not disrupted by any of the deleted y<sup>+</sup>LAT-1 proteins. The localization of the small deletions is similar to the wild type, whereas proteins lacking more than 60 amino acids remain cytoplasmic. Transfection of C-terminally deleted y<sup>+</sup>LAT-1s results in reduction of y<sup>+</sup>LAT-1-4F2hc expressing cells compared to transfection of wild-type y<sup>+</sup>LAT-1. In addition, expression of truncated y<sup>+</sup>LAT-1 affects cellular viability, which may be due to scavenging of 4F2hc by the mistargeted cytoplasmic y<sup>+</sup>LAT-1, leading to shortage of 4F2hc in the plasma membrane and reduced cell proliferation compared to the cells expressing normally targeted 4F2hc.

## P12.106

## Gene expression profiling of haematological and immunological deficiencies in lysinuric protein intolerance (LPI) patients

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Lysinuric protein intolerance (LPI; MIM222700) is a rare autosomal recessive disorder caused by a defect of cationic amino acid transport in the small intestine and kidney tubules. All Finnish patients share the same homozygous mutation c.1181-2A>T (c.859-2A>T) in the SLC7A7 gene. Altogether 51 mutations have been found worldwide but the Finnish founder mutation has not been detected in any other population. The main symptoms of LPI comprise protein aversion after weaning, failure to thrive, muscle hypotonia, osteoporosis and hepatosplenomegaly. However, some findings vary markedly even in the same family, and may include severe renal and pulmonary complications, such as end-stage renal disease and alveolar proteinosis. Some patients suffer from normochromic anaemia with poikilocytosis and anisocytosis and immunological problems with leukopenia and deficiencies in T- and B-cell functions.

We have used microarray technology and quantitative real-time PCR to find out which other genes than SLC7A7 may affect the phenotype of the patients. The gene expression profiles were obtained from the peripheral whole-blood cells. We found systematic up-regulation of genes encoding proteins participating in erythropoiesis, heme synthesis, erythrocyte membrane structure, transport, enzymatic functions and blood group antigens. Down-regulated genes encoded e.g. interleukins, interleukin receptors, chemokines, regulators of complement activity and histocompatibility complex proteins. These differentially expressed genes may, indeed, light the background of haematological and immunological problems observed in LPI patients.

## P12.107

Clinical and genetic heterogeneity of Mal de Meleda: exclusion of genetic linkage to the *ARS* gene in a Tunisian family

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**Background.** Mal de Meleda (MdM) is a rare form of palmoplantar keratoderma (PPK) with autosomal recessive transmission. It is characterized by diffuse erythema and hyperkeratosis of the palms and soles. Recently, mutations in the *ARS* (component B) gene (*ARS*, MIM: 606119) on chromosome 8q24.3 have been identified in families with this disorder.

**Objective.** Clinical and genetic investigation of a consanguineous family from Northern Tunisia with transgressive palmoplantar keratoderma closely resembling the Mal de Meleda phenotype.

**Methods.** A family with six members, among them two were affected individuals, was recruited for this study. Mutation screening of the *ARS* gene was performed by direct sequencing and haplotype analysis using 2 microsatellite markers (D8S1836 and D8S1751) flanking the *ARS* gene.

**Results.** No mutation was found in the coding region and exon-intron junctions of the *ARS* gene within the explored family. This genetic exclusion of the *ARS* gene was confirmed by haplotype analysis and Lod score calcul.

**Conclusion.** This is the second Tunisian Family with "MdM like" phenotype. Our findings give further evidence for the molecular heterogeneity of the MdM phenotype in North African population.

## P12.108

Quantitative sequence analysis of *FBN1* premature termination codons provides evidence for incomplete NMD in leukocytes

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In order to assess the pathogenic effects of mutations, we improved, evaluated, and used Sanger sequencing for quantification of SNP variants in transcripts and gDNA samples. This improved assay resulted in highly reproducible relative allele frequencies (e.g. for a heterozygous gDNA  $50.0 \pm 1.4\%$ ,  $P=0.05$ , and for a missense mutation-bearing transcript  $46.9 \pm 3.7\%$ ,  $P=0.05$ ) with a lower detection limit of 3-9%. It provided excellent accuracy (e.g. for a duplicated gDNA  $66.6 \pm 2.2\%$ ,  $P=0.05$ ) and linear correlation between expected and observed relative allele frequencies. This sequencing assay, which can also be used for the quantification of CNVs, methylations, mosaicism, and DNA pools, enabled us to analyze transcripts of the *FBN1* gene in fibroblasts and blood samples of patients with suspected Marfan syndrome not only qualitatively but also quantitatively. We present a total of 19 novel and 18 known *FBN1* sequence variants leading to a premature termination codon (PTC), 26 of which we analyzed by quantitative sequencing both at gDNA and cDNA levels. The relative amounts of PTC-containing *FBN1* transcripts in fresh and PAXgene-stabilized blood samples were significantly higher ( $33.0 \pm 3.9\%$  to  $80.0 \pm 7.2\%$ ,  $P=0.05$ ) than those detected in affected fibroblasts with inhibition of nonsense-mediated mRNA decay (NMD) ( $11.0 \pm 2.1\%$  to  $25.0 \pm 1.8\%$ ,  $P=0.05$ ), while in fibroblasts without NMD inhibition no mutant alleles could be detected. These results provide evidence for incomplete NMD in leukocytes and have particular importance for RNA-based analyses not only in *FBN1* but also in other genes.

## P12.109

Mutation analyses of *MYH11*, *ACTA2*, *TAGLN*, and *TGFBR3* in patients with suspected Marfan syndrome

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Marfan syndrome (MFS) is an autosomal dominant disorder of connective tissue, which displays variable manifestations in the skeletal, ocular, and cardiovascular systems. Patients with suspected MFS referred to us for molecular genetic testing carry the disease-causing mutation in *FBN1*, *TGFBR1*, and *TGFBR2* in the majority of cases. Negative genetic testing results can be due to assay limitations or because the disease-causing mutation is located in another gene. Indeed, mutations in *MYH11* and *ACTA2* have recently been associated with familial thoracic aortic aneurysms and dissections (TAAD), which overlap with cardiovascular features of MFS, and *TAGLN* has been predicted in silico as a candidate gene for TAAD. Furthermore, considering the TGF-beta pathway, one could also expect mutations in the *TGFBR3* gene. Here, we analysed *MYH11*, *ACTA2*, *TAGLN*, and *TGFBR3* in patients with suspected MFS in whom previous mutation analyses of *FBN1* and/or *TGFBR1* and *TGFBR2* detected no mutation. Exon-by-exon gDNA sequencing of 24 (*MYH11*), 89 (*ACTA2*), 46 (*TAGLN*), and 12 (*TGFBR3*) unrelated patients revealed two putative pathogenic mutations in *MYH11* (p.Leu1752\_Glu1755dup and p.R1794Q) as well as one missense mutation in *ACTA2* (p.I371T). Screening of *TAGLN* and *TGFBR3* revealed no putative pathogenic sequence changes. Although the number of patients analysed in this study is still small, our results indicate that screening for *MYH11* and *ACTA2* mutations should be considered in patients with suspected MFS in whom mutation analyses of *FBN1*, *TGFBR1*, and *TGFBR2* revealed no disease-causing mutation, in particular in patients with cardiovascular complications lacking skeletal features of MFS.

## P12.110

## Identification of genome wide targets of the UPF3B dependent nonsense-mediated mRNA surveillance pathway in patients with mutations in UPF3B using exon array

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Non-sense mediated mRNA decay (NMD) is a universal RNA surveillance pathway that among other functions degrades mRNAs bearing premature termination codons (PTC). We recently showed mutations in UPF3B, an important member of this pathway, caused syndromic and nonsyndromic mental retardation (MR). To assess the impact of UPF3B null mutations and identify relevant genes regulated by NMD, we performed expression profiling using RNA isolated from control and patient lymphoblastoid cells using Affymetrix Human Exon 1.0 ST arrays. Compared to controls, 633 genes were significantly de-regulated in patients (30% up, 70% down, false discovery rate = 10%), including down-regulation of UPF3B. Hence, NMD is only partially compromised in the absence of UPF3B as its own PTC containing mRNA is degraded. Comparison with previous microarray studies from UPF1, UPF2 or UPF3B knock down cell lines in human, fly and yeast generated minimal overlap. Such low correspondence indicates these studies do reflect the situation of a real knock out or reflects differences in the tissue types and platforms used. Among the significantly de-regulated genes identified in patients with mutations in UPF3B, ARHGAP24 was highly up-regulated. ARHGAP24 is a negative regulator of Rho-GTPase and has role in regulating cell polarity. We show that PC12 cells over expressing ARHGAP24 failed to differentiate into neuronal-like cells upon treatment with nerve growth factor. We have identified the bona-fide targets of the UPF3B dependent NMD pathway and suggest up-regulation of ARHGAP24 contributes to the phenotypes seen in patients with MR due to mutations in UPF3B.

## P12.111

**Recessive congenital methaemoglobinaemia type II: a novel mutation in the NADH-cytochrome b5reductase gene in a Russian patient**

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Hereditary methemoglobinemia is an autosomal recessive disorder caused by NADH-cytochrome b5 reductase (cytb5r) deficiency. Two forms of cytb5r are known, a soluble form and a membrane-bound form, and are localized in different cellular compartments. Hereditary methemoglobinemia has been classified into two types, an erythrocyte type (type I) and a generalized type (type II). Type I is characterized clinically by a single symptom, cyanosis, and biochemically by a deficiency of the red cell-soluble form of the enzyme. In type II, the cyanosis is accompanied by neurological impairment, mental retardation and reduced life expectancy. Type II is characterized by deficiency the soluble and the membrane-bound forms of the enzyme in various tissues of patients. The cytb5r gene (*DIA1*) is 31-kb long, contains 9 exons, and has been localized to chromosome 22q 13-qter.

We have investigated family with methemoglobinemia type II from the Chechen Republic of the Russian Federation. There were 5 children in this family. First, second and fourth child was not affected. Third and fifth child suffered from cyanosis and neurological impairment. Third child dead at the age of four years. We have investigated fifth child at the age of 6 month and found a novel mutation (c.339insC) in exon 5 of the gene *DIA1* in homozygote. This insertion leads to appearance premature stop codon.

## P12.112

**Improved molecular diagnostics for patients with respiratory chain complex deficiency**M. Biste<sup>1</sup>, F. Madignier<sup>1</sup>, P. Freisinger<sup>2</sup>, B. Rolinski<sup>3</sup>, J. Mayr<sup>4</sup>, M. Tesarova<sup>5</sup>, R. Horvath<sup>3</sup>, W. Sper<sup>4</sup>, T. Meitinger<sup>1</sup>, H. Prokisch<sup>1</sup>;

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**Aim:** Isolated respiratory chain complex I (RCCI)-deficiencies are the most common form of mitochondrial diseases (round 25%). Clinically, the patients present a heterogeneous spectrum which can be multi-systemic (e.g. neonatal lactic acidosis, Leigh syndrome) or with distinct symptoms (e.g. ataxia, myopathy). RCC I is composed of 45 different subunits. The high number of genes involved is making the search for the molecular basis of RCCI deficiency difficult. In routine diagnostics the causal mutations can be identified in less than 10% of the pediatric patients with RCCI-deficiency.

**Methods:** A high-throughput molecular genetic screen for the RCCI encoding genes was established. 92 families with isolated RCCI-deficiency and previous exclusion of common mtDNA mutations were investigated by DNA melting profile analysis using an Idaho LightScanner. To analyze genotype-phenotype correlations a clinical questionnaire was developed based on the guidelines issued by the working group on pediatric metabolic disorders.

**Results:** We have screened 59 genes coding for the subunits and assembly factors of RCCI. Causative mutations have been identified in 16% of patients which were inconspicuous in routine diagnostics. A single variant was identified in 30% of additional samples and in 54% of samples no mutations have been found yet. The analysis shows typical clinical patterns correlated with mutations in specific genes.

**Conclusion:** Molecular genetic diagnostics of RCCI-deficiency was improved and prenatal diagnostics can be offered. Genotype-phenotype correlations will enable more efficient diagnosis and allow predictions on disease course.

## P12.113

**A novel mutation in the mitochondrial ATPase 8 gene in a patient with leukodystrophy**E. Mkaouar<sup>1</sup>, F. Kammoun<sup>2</sup>, I. Chamkha<sup>1</sup>, I. Hsairi<sup>2</sup>, C. Triki<sup>2</sup>, F. Fakhfakh<sup>1</sup>;

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Mitochondrial DNA defects were known to be associated with a wide spectrum of human diseases and patients might present with a wide

range of clinical features in various combinations. In the present study, we described a patient with a form of leukodystrophy showing psychomotor and neurodevelopmental delay, mild hyperintensity of posterior periventricular white matter, spastic paraparesis, generalized clonic seizures and congenital deafness. He also suffered from a severe tetraparesis, with central blindness and swallowing difficulty. Brain MRI showed involvement of the interpeduncular nucleus and central tegmental tract, white matter abnormalities and cerebellar atrophy. A whole mitochondrial genome screening in this patient revealed the presence of 19 reported polymorphisms and an undescribed A to G heteroplasmic mutation at nucleotide 8411 (M16V) affecting a highly conserved region of the mitochondrial ATPase 8 protein. This mutation could be associated to the disease in the tested patient who belongs to haplogroup U.

## P12.114

**Molecular genetic exploration-diagnostic tool for chronic granulomatous disease. Case report**M. Serban<sup>1</sup>, M. Bataneant<sup>1</sup>, C. Jinca<sup>1</sup>, D. Mihailov<sup>1</sup>, M. Puiu<sup>1</sup>, L. Dehelean<sup>1</sup>, L. Morodi<sup>2</sup>;

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**Introduction:** Chronic granulomatous disease (CGD) is a heterogeneous congenital immunodeficiency characterized by a profound defect in the burst of oxygen consumption. Beside the genuine CGD there are a lot of CGD-like syndromes, therefore a molecular genetic investigation is mandatory for a definite diagnosis, decisive for an appropriate therapeutic decision.

**Case presentation:** We present the case of a 8 years old boy with a history of recurrent episodes of fever, adenophlegmons and pulmonary infections since the age of 3 weeks. Hepatosplenomegaly as well as pulmonary and vertebral aspergillosis with destruction of thoracic vertebrae D5-D7 characterized the clinical picture at admittance in our hospital. The suspicion of X-linked CGD was confirmed by absent respiratory burst and the presence of the genetic mutation (4. exon, c.271C>T, p.R91X). Despite prolonged therapy, the patient continued to present pulmonary and vertebral aspergillosis. Due to the presence of an HLA-compatible brother, matched related PBSCT was performed under continuous antifungal treatment. Chimerism analysis showed complete donor chimerism of granulocytes, monocytes, NK cells and CD19 lymphocytes and 85% and 96% donor chimerism of CD4 and CD8 lymphocytes respectively. Respiratory burst performed showed significant improvement. At 7 months after the HSCT, under antifungal maintenance therapy, the patient is in good clinical condition with full donor chimerism and significantly improved radiologic findings on the chest x-ray.

**Conclusion:** Molecular genetic exploration proved the diagnosis of CGD in our case. Its genetic marker 4 exon, c.271C>T, p.R91X was clinically expressed in a life-threatening infection, justifying a successfully undertaken hematopoietic stem cell transplantation.

## P12.115

**Molecular mechanism underlining genetic defects in Incontinentia Pigmenti**M. Paciolla<sup>1</sup>, A. Pescatore<sup>1</sup>, F. Fusco<sup>1</sup>, J. Gauteron<sup>2</sup>, M. G. Miano<sup>2</sup>, G. Courtois<sup>2</sup>, M. V. Ursini<sup>1</sup>;

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Incontinentia Pigmenti (IP) is an X-linked dominant genodermatosis, lethal in male, caused by mutations in the Xq28 *NEMO* gene. *NEMO* is the essential subunit of the kinase complex IKK, required for the activation of NF-κB canonical pathway. The most frequent IP mutation (80%) is a recurrent genomic exons 4-10 *NEMO* deletion. In addition, about 39 small mutations scattered along *NEMO*, have been reported.

In order to unravel molecular mechanism that underlines the alteration of NF-κB activation in the pathology, we performed an analysis of the *NEMO* mutations associated to severe forms of IP. In particular, we demonstrated that the A323P presents an impairment of (K63-) polyubiquitination, which resulted from a defective interaction with TRAF6. This analysis allowed us to define the critical lysines residues of *NEMO*, target of TRAF6, that are required for proper response to multiple NF-κB activation signals, such as IL-1 and LPS. Starting from

those evidences, we established that other IP-associated NEMO mutations, such as E57K and DeltaK90 showed a defective interaction with TRAF6. The sum of this observation supports the view that multiple signal modifications, mainly polyubiquitination, that converge at NEMO are necessary events in IKK activation whose perturbation may cause human pathophysiology.

#### P12.116

#### Conventional mutations are associated with a different phenotype than polyglutamine expansions in spinocerebellar ataxias

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Autosomal dominant cerebellar ataxias comprise a wide spectrum of diseases with different clinical/neuropathological profiles. At least 30 responsible loci (SCA) have been mapped. Nucleotide repeat expansions have been identified as responsible for the disease in 9 genes including those caused by polyglutamine-coding (CAG)n repeat expansions in the SCA1-3,6,7 and 17 genes and rare forms caused by non-coding repeats in the SCA8,10 and 12 genes. More recently, conventional mutations were reported in SPTBN2/SCA5, TTBK2/SCA11, KCNC3/SCA13, PRKCG/SCA14, ITPR1/SCA15/16, FGF14/SCA27, AFG3L2/SCA28 as well as in the puratrophin gene.

The relative prevalence of the SCA genes and their associated phenotype was investigated in 826 index patients from families with a dominant transmission of the disease collected from 1990 to 2008 in the Pitié-Salpêtrière university-hospital in Paris or through clinical national networks using standardized clinical charts.

The most frequently mutated genes were SCA3 (20.4%), SCA2 (9.7%), SCA1 (7.7%), SCA7 (5.7%) and SCA6 (1.8%). Missense mutations in SCA14 (1.8%), SCA28 (1.6%), SCA13 (1.2%) and SCA5 (0.8%) were less frequent. We found SCA17 (0.2%) and SCA12 (0.2%) to be very rare, while no cases of SCA10, 27 or puratrophin were identified. In subclinical selections, heterozygous deletions in SCA15/16 (4/76) and a non-sense mutation in SCA11 (1/77) were also detected.

Genotype-phenotype correlations showed that CAG repeat expansion diseases shared a rapidly progressive and severe disease course with onset in the thirties. On the contrary, the clinical picture associated with conventional mutations in the recently identified genes was milder despite the frequent presence of marked cerebellar atrophy on MRI.

#### P12.117

#### Nance-Horan syndrome and X-linked cataract are allelic disorders

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Nance-Horan syndrome (NHS) is an X-linked developmental disorder characterised by congenital cataract, dental anomalies, facial dysmorphism, and in some cases, mental retardation. Protein truncation mutations in a novel gene (*NHS*) have been identified in patients with this syndrome. Isolated X-linked congenital cataract (CXN) has been mapped to chromosome Xp22.13, which encompasses the *NHS* locus, however, no mutations were identified in the *NHS* gene. In this study we describe a clinical and molecular analysis of 7 NHS and 2 CXN families. We have identified five new protein truncation mutations and a large deletion encompassing the majority of the *NHS* gene in NHS families. Two CXN families, who were negative for mutations in the *NHS* gene, were further analysed using array comparative genomic hybridisation (CGH). We show, for the first time, that these diseases

are indeed allelic. Interestingly, all mutations in the *NHS* gene causing Nance-Horan Syndrome are protein truncating mutations. In contrast, two X-linked cataract families have different copy number variations of the *NHS* gene; an intragenic deletion and a complex duplication-triplication rearrangement. We suggest that both of these mutational events lead to altered transcriptional regulation of the *NHS* gene leading to a milder phenotype than Nance-Horan syndrome. Analysis of the position and sequences of the breakpoints pinpoints regulatory sequences for *NHS* gene expression, and also potential molecular mechanisms for non-recurrent non-homologous recombination. Our data show the importance of different mutational mechanisms leading to different severities of disease.

#### P12.118

#### Possible involvement of *N*-acetyltransferase 2 in development of endometriosis

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Endometriosis is a common disease defined as a growth of endometrial tissue outside the uterine cavity that often results a vast array of gynaecological problems including dysmenorrhoea, pelvic pain, infertility. Basic aetiology and pathogenesis of this condition isn't clearly enough. Endometriosis is regarded as one of the multifactorial diseases caused by an interaction between the environment and multiple genes.

*N*-acetyltransferase 2 is the enzyme realized *N*-acetylation and biotransformation of xenobiotics. NAT2 slow genotypes affect detoxification function and might increase the risk of the disorder. The present study was carried out to investigate if polymorphisms of *NAT2* are useful markers for predicting endometriosis susceptibility.

DNA was extracted from blood of 86 patients with reliable diagnosis of endometriosis and 53 healthy women (control group). *NAT2* gene polymorphism was detected in five polymorphic sites: c.341T>C, c.481C>T, c.590G>A, c.803A>G, c.857G>A. PCR-PFLP analysis was applied to detect the missense substitution in nt position 590 and MLPA for others. The relative frequencies of the polymorphisms between both groups were compared.

The proportions of individuals homozygous for c.341C, c.590A, and c.803G were 18.6 and 11.3%, 7.0 and 5.6%, 19.8 and 9.4% in the endometriosis and control groups accordingly ( $P > 0.05$ ). There were no individuals homozygous for c.857A in both groups. The proportion of c.481T homozygous individuals was 30.2% in patients and 7.5% in controls that corresponded to significantly difference between two groups ( $P = 0.001$ ). These results show the significant impact of *NAT2* gene polymorphism in position 481 in development of endometriosis.

#### P12.119

#### *NPHS2* and *WT1* gene mutations in Greek children with steroid resistant nephrotic syndrome (SRNS)

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Several genes are implicated in the pathogenesis of autosomal recessive SRNS, including *NPHS2* (encoding podocin) and *WT1* (transcription factor Wilm's tumor-1). The presence of mutations in the 8 exons of the *NPHS2* gene, along with "hot-spot" exons 8 and 9 of the *WT1* gene, were investigated (direct sequencing) in 27 SRNS patients (2-18 years), including 8 familial (3 families) and 19 sporadic cases.

*NPHS2* analysis revealed pathogenic genotypes in 3/19 sporadic patients: homozygosity for R138Q (c.413G>A), homozygosity for R168H (c.503G>A) and compound heterozygosity for R229Q (c.686G>A) in trans to A295T (c.883G>A). The novel A295T (exon 8) is predicted to be pathogenic by in silico assessment. Amongst familial cases, 3 patients were heterozygous for R229Q without a second *NPHS2* mutation. Additionally, several known polymorphisms (IVS3-46C>T, IVS3-21C>T, IVS7+7A>G, S96S, A318A, and L346L) were found in both patients and 100 unaffected controls with equal allele frequencies. A novel intronic *NPHS2* variant (IVS3-17C>T), was found in 2 related

patients, but no controls, and is under further investigation. Four patients carried de-novo *WT1* mutations, 3 had previously characterized mutations (1 with IVS9+5G>A, and 2 with R394W,) and the fourth had a novel mutation R366H, predicted to be pathogenic in silico. Karyotype analysis showed XX in 3 patients, consistent with the female phenotype, but one (with R394W), was XY and had complete gonadal dysgenesis. Thus 7/19 sporadic SRNS patients, (but no familial cases) had *NPHS2* or *WT1* mutations, indicating that molecular investigation of these genes is useful to support definitive diagnosis and management of pediatric SRNS..

#### P12.120

#### Genotype-phenotype correlations in patients with hereditary neurodegenerative diseases

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Neurodegenerative diseases (ND) are characterized by progressive nervous system (NS) dysfunction, associated with mutation in genes leads to atrophy of the affected central or peripheral NS structures. To summarize our experience in the field of neurogenetics we provide the investigation of mutations associated with various NS dysfunction in patients with Huntington's disease (HD) and Charcot-Marie-Tooth (CMT) disease.

HD is a common disorder of central NS caused by expansion of CAG-repeats in the *IT15*. CMT is a heterogeneous group of peripheral NS diseases with more than 20 involved loci. The most common types of CMT are due to mutations in *PMP22* and *Cx32*.

Analysis of CAG-, CCG- and del2642 of *IT15* gene polymorphisms has been performed in patients with HD. We have provided the screening of *PMP22* duplications/deletion and *Cx32* point mutations in CMT patients. 35 HD-probands had expanded allele of *IT15* (39-52 CAG-repeats). The significant differences in sex-determined instability of CAG-repeats inheritance have been revealed. The association between del2642 and the age of HD onset has been analyzed. *PMP22*-duplications were found in 21 CMT-families. We also detected the heterozygote *PMP22* gene deletion in two patients with specific HNPP-phenotype. Mutation screening of *Cx32* revealed 2 brothers with Arg22Gln mutation. Our data showed the association between different types of *IT15*, *PMP22* and *Cx32* genes mutations and specific abnormalities of the NS. Obtained data would be useful for better understanding of ND pathogenesis and for providing new individualized therapy for curing of neurological disorders.

#### P12.121

#### Identification and characterization of mutations causing Niemann-Pick disease types A/B in Spanish patients

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Niemann-Pick disease type A/B (NPD A/B) is an autosomal recessive lysosomal storage disorder caused by acid sphingomyelinase (ASM) deficiency due to mutations in the *SMPD1* gene. Type A NPD is the severe neurological form whereas type B NPD patients have no neurological manifestations. In this work, we present a molecular analysis of 19 Spanish patients and 2 from Maghreb, 8 with type A and 13 with type B NPD. All mutant *SMPD1* alleles were identified, including 17 different mutations, 10 of which were novel: c.503G>A (p.W168X), c.939C>A (p.Y313X), c. 1100A>G (p.Y367C), c.1400A>C (p.Y467S), c.1445C>A (p.A482E), c.1456A>G (p.T486A), c.1159delC (p.R387VfsX7), c.1169\_1171delTCT (p.F390del), c.1257+4\_1257+7delAGGG, and c.1774\_1776delACT (p.T592del). The only frequent mutations in the 21 NPD patients were c.1823\_1825delGCC (p.R608del) (38%) and c.1445C>A (p.A482E) (9%). For most of the mutations a good correlation between genotype and phenotype could be established and, in particular, the p.R608del-type B association was confirmed. Six of the mutations found in Spanish patients, and two other mutations for comparison, were expressed *in vitro* to establish their residual enzymatic activities.

All mutant alleles were confirmed to be disease-causing due to their low enzyme activity, although western blot analyses showed that a normal amount of protein was synthesized. The mutation c.1257+4\_1257+7delAGGG, which affects a non-canonical donor splice site, was analysed at the RNA level. Only aberrant mRNAs, corresponding to previously reported minor *SMPD1* transcripts, which do not code for functional enzymes, were produced by this allele. This study is the first exhaustive mutational analysis of Spanish Niemann-Pick A/B disease patients.

#### P12.122

#### The Evaluation of Clinical Selection Criteria in NSHL Molecular Results

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Since January 2001 we have studied more than 1000 subjects among deaf patients, family members and deaf/carrier partners.

The majority of our patient's population has been recruited through the Genetic Service of the O.R.L. Department of our Hospital in Milan since 2001 and through the O.R.L. Service in Piacenza starting from 2004. The 658 subjects afferent to our Hospital were affected by neurosensory deafness with various degrees (from mild to profound) of hearing loss (HL), while the 131 patients from Piacenza Hospital had a HL degree ranging from severe to profound.

All patients were analysed for mutations of the entire *Cx26* gene (GJB2), and for the  $\Delta$ (GJB6-D13S1830) deletion. The analysis of the deafness-causing A1555G substitution in mitochondrial (mt)DNA was carried out in our cohort of patients while, usually, in the second group this analysis is not requested. In order to compare the two different criteria of clinical selection and the concerning molecular results, we performed the mtDNA analysis in all the subjects with only one or without *Cx26* mutations. In the first group we found a lower percentage of positive *Cx26/Cx30* cases in comparison to the second group (25.8% vs. 53.4%), but a higher percentage of dominant mutations (4.7% vs. 1.5%); the mtA1555G was found only in the first group of the affected patients (3%). We conclude that clinical selection based on the severity of HL is quite relevant to obtain high percentage of *Cx26* positive, but may allow the missing of uncommon GJB2 genotypes and mt mutations.

#### P12.123

#### Frequency of 35delG mutations in cochlear implant recipients

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Objective: Hearing impairment is the most common sensory disorder, present in 1 of every 500 newborns. Nonsyndromic sensorineural hearing loss(NSHL) is inherited in a predominantly autosomal recessive manner in up to 70% of cases. It is also an extremely heterogeneous trait. The gene more often involved is GJB2, encoding the protein Connexin 26. In most populations a single mutation, 35delG, accounts for most cases of NSHL.

Methods: 90 patients receiving cochlear implants were ascertained through research centre of ENT & head and neck surgery .After genetic counseling for them DNA isolated from the peripheral blood, all patients were molecularly evaluated for the presence of the 35delG mutation by ARMS /PCR.

Result: We investigated 90 patients , 44.9% male and 55.1% female , that 90% of them were < 10 years old that receiving cochlear implant

70.8% of these patients were born on consanguine family and 12.4% of them were syndromic hearing loss.

Among these patients 86.5% were normal, 10.1 % homozygote and 3.4% heterozygote for 35delG mutation.

Conclusion: The most frequent genes implicated in autosomal recessive nonsyndromic hearing loss are GJB2, which is responsible for more than half of cases. In our study, there was a significant relationship between consanguineous marriage in our evaluated group and express of NSHI (PV#0/00 and O.R= 3.43), but there was not any significant relationship between inheritance pattern and consanguineous marriage with 35delG mutation. These data can help genetic counselor and otolaryngologist for setting priority in evaluation, prevention and even treatment in these patients.

#### P12.124

#### De novo mutations in STXBP1 cause early infantile epileptic encephalopathy

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Early infantile epileptic encephalopathy with suppression-burst (EIEE), also known as Ohtahara syndrome, is one of the most severe and earliest forms of epilepsy. Using array-based comparative genomic hybridization (aCGH), we found a *de novo* 2.0-Mb microdeletion at 9q33.3-q34.11 in a female EIEE patient. Mutation analysis of candidate genes mapped to the deletion revealed that four unrelated EIEE patients had heterozygous missense mutations in *syntaxin binding protein 1* (STXBP1). STXBP1 (also known as MUNC18-1) is an evolutionarily conserved neuronal Sec1/Munc-18 (SM) protein, which plays an essential role for synaptic vesicle release in multiple species. Circular dichroism (CD) melting experiments revealed that a mutant protein was significantly thermolabile compared to the wild type. Furthermore, binding of the mutant protein to syntaxin was impaired. These findings suggest that haploinsufficiency of STXBP1 causes EIEE. Following doctors are highly appreciated: Drs. Mitsuhiro Kato (Yamagata University School of Medicine), Hitoshi Osaka (Kanagawa Children's Medical Center), Jun Tohyama (Nishi-Niigata Chuo National Hospital), Katsuhisa Urano (NHO Yamagata National Hospital), Satoko Kumada (Tokyo Metropolitan Neurological Hospital).

#### P12.125

#### New mutations in the CXORF5 (OFD1) gene and the influence of X-inactivation on the phenotype in patients with Type I Orofaciodigital Syndrome

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Thirteen forms of Orofaciodigital Syndrome (OFDS) have been described, however CXORF5 (Xp22.3-p22.2) is currently the only known causative gene, in which mutations cause OFD type I (OFD1). OFD1 is characterized by malformations of the face, oral cavity, and digits and is transmitted as an X-linked dominant condition with lethality in males. There may be central nervous system involvement in as many as 40% of cases, and polycystic kidney disease seems to be specific to OFD1. We have performed mutation analysis via DNA sequencing in 27 sporadic and two familial cases of suspected OFD1. Fourteen mutations, nine of which have not previously been described, were found in the index patients. Five of the mutations (36%) are predicted to affect splicing. Mental retardation has previously been associated with mutations in CXORF5 exons 3, 8, 9, 13 and 16. We have found a new splice mutation in intron 1, c.13-10T>A, in a mother and her two daughters, with greatly diverging phenotypes, especially with regard to cognitive ability. X-inactivation studies in lymphocytes showed preferential inactivation of the mutation allele in the mildly affected mother (normal intelligence) and relatively mildly affected daughter, whereas both alleles appeared to be equally active in the severely retarded daughter. Reverse transcription PCR and sequencing in lymphocytes revealed the markedly increased presence of an extra, larger transcript which includes intron 1, in the most severely affected daughter. Our results suggest that the pattern of X inactivation has a greater effect on the brain phenotype than the type of mutation.

#### P12.126

#### Mutational spectrum of the COL1A1 and COL1A2 genes in Spanish patients with Osteogenesis Imperfecta

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Osteogenesis Imperfecta (OI) is a group of disorders characterized by bones that break easily. Clinical features are very heterogeneous

and up to seven clinical types have been described. OI is predominantly inherited in an autosomal dominant manner although recessive forms have been described. Dominant forms are caused by mutations in either COL1A1 or COL1A2 genes, located on 17q21-22 and 7q22 chromosomal regions respectively. In this work we report our results and experience in the genetic diagnosis of Osteogenesis Imperfecta in a wide cohort of patients coming from several Spanish hospitals. Mutational analysis of both COL1A1 and COL1A2 genes has been performed by double-strand sequencing. Results are discussed on the basis of inter and intragenic mutational distribution, type of mutation, aminoacid residues, familial implications and genotype-phenotype correlations. We highlight the elevated percentage of mutations not previously reported (more than 50%) identified in our population. A high expertise and experience must be required for the interpretation of nucleotide changes identified in genes with a high rate of *de novo* and novel (not yet reported) mutations, especially, in paediatric patients because potential social and legal implications and patients with a reproductive interest. Mutational spectrum of COL1A1 and COL1A2 genes depicted in our population provides an interesting epidemiologic and pathogenic information about Osteogenesis Imperfecta.

#### P12.127

#### Distinct OI Phenotype Caused by COL1 C-proteinase Site Mutations

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Osteogenesis imperfecta (OI) is often caused by mutations in the type I collagen genes. Mutations in the type I procollagen C-propeptide cleavage site are of interest because they disrupt a processing step. We identified two children with mild OI who had cleavage site mutations in COL1A1 (P1:  $\alpha 1(I)D1041N$ ) or COL1A2 (P2:  $\alpha 2(I)A1029T$ ). P1 DEXA z-score and pQCT vBMD were +3, contrasting with radiographs demonstrating osteopenia and os-in-os vertebrae, and histomorphometry revealing increased bone remodeling, without a mineralization defect or signs of osteosclerosis. P2 had a DEXA z-score of 0, gracile long bones with radiographic osteopenia, and decreased BV/TV and increased BFR without a mineralization defect on histomorphometry. FTIR imaging analysis in both cortical and trabecular bone confirms that P1 and P2 have elevated mineral/matrix and collagen maturity compared to age-matched controls or a proband with classical OI. Steady-state collagen electrophoresis showed slight overmodification of  $\alpha 1(I)$  and  $\alpha 2(I)$  in cell layers of both probands, with a slight baseline delay in P1. Chain incorporation was normal in P1 and slightly delayed in P2. Pericellular processing of P1 was delayed, with increases in both p $\alpha 1$  and p $\alpha 2$ , while P2 had increased p $\alpha 2$  and p $\alpha 2$  and normal processing kinetics. Together with an adult with an  $\alpha 1(I)A1040T$  substitution (Int Conn Tis 82S1: CC01), our cases suggest that defects in p $\alpha 1(I)$  processing lead to high childhood BMD possibly due to increased bone mineral content, with signs of osteopetrosis occurring subsequently. P $\alpha 1(I)$  cleavage appears crucial to C-propeptide processing, while defective p $\alpha 2(I)$  cleavage occurs after p $\alpha 1(I)$  processing.

#### P12.128

#### Pelizaeus-Merzbacher Disease - different molecular defects result in various clinical picture?

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Pelizaeus-Merzbacher disease (PMD; OMIM 312080) is a rare, severe dysmyelination brain disorder caused by mutation in the X-linked gene PLP1. PMD typically manifests in infancy or early childhood with nystagmus, hypotonia, and cognitive impairment; the findings progress to severe spasticity and ataxia; life span is shortened. PLP1 protein is exceptionally well-conserved in mammals showing nearly no poly-

morphism and all types mutation have a discernible impact in human. The classes of mutations causing PMD are mainly duplications of the whole gene and point mutations.

The purpose of this study is to analyse types of the PLP1 gene mutations among Polish PMD patients and compare the clinical picture in the context of the causative mutation.

The molecular analysis of the PLP1 gene was performed according to general guidelines starting with gene dosage screening (MLPA method) followed by direct sequencing for patients without rearrangements. Any type of PLP1 changes were described in 37% of probands. The duplication of all PLP1 exons was confirmed for four patients presenting different clinical picture (except brothers). We expected that the gene dosage might be modified by different size of duplication. To define more precisely the duplicated region aCGH was performed.

The impact of discovered missense mutations on PLP1 protein structure and functional changes were analysed by *in silico* modelling.

Analyses comprise the group of PMD male patients; four (2 unrelated, 2 brothers) with duplication of the whole PLP1 gene, two patients (brothers) with nonsense mutation in Ex3 and two unrelated patients with missense mutations in different exons

#### P12.129

#### Mutations spectrum in the STK11 gene in Polish Peutz- Jeghers syndrome patients

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Peutz-Jeghers syndrome (PJS) is rare, genetically conditioned disease. PJS is herited in autosomal dominant manner and is characterized by occurrence of hamartomatous polyps. The hamartomatous polyps are manifested during second or third decade of life. Occurrence hamartomatous polyps in PJS may cause of many gastrointestinal discomforts. Although in PJS patients the risk of malignant transformation is lower then others hereditary neoplastic disease, an increased risk to development malignancies such as the pancreas, the breast, female and male reproductive organs is observed. The second characteristic manifestations of PJS are brown, dark or blue spots. PJS is caused by mutations in the STK11 on chromosome 19. STK11 gene encodes a serine/threonine protein kinase participating in very important cell signalling pathways. We present study considering 20 patients diagnosed with PJS. PJS diagnosis was based on presence of two or more polyps, or one polyp and typical pigmented lesions, or one polyp and a family history of PJS. Mutations screening analysis encompassing SSCP, HA and direct sequencing of the LKB1 gene are revealed five mutations and one polymorphism. These mutations are located in different position in gene (1, 2, 7 exons). With the Multiplex Ligation-dependent Probe Amplification (MLPA) - assay we detected additional genomic mutations. For our screening we used the SALSA P101 STK11 kit which contains MLPA probes for most STK11 exons. In four patients we identified exonic deletions or duplications range from one to five exons

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#### P12.130

#### Molecular analysis of most common mutations in Phenylalanine hydroxylase gene in Iranian population

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Phenylketonuria (PKU) is the most prevalent disorder of amino acid metabolism. It is one of the most important preventable causes of mental retardation. Incidence of PKU in Iran has been estimated at 1 in 3600-4000 births. The same is true of Turkey. PKU is an autosomal recessive disorder and it is caused by a deficiency of hepatic phenylalanine hydroxylase enzyme. To date several hundred mutations caus-

ing PKU have been characterized in the PAH gene.

The aim of this study is to assess the prevalence of PKU mutations in Iranian population. For this purpose, 150 unrelated patients with classic PKU (300 alleles) were screened for 10 mutations (IVS10-11g>a, R252W, R261X, R261Q, IVS11nt1, R408W, R408Q, L333F, 364delG and S67P) using polymerase chain reaction-restriction fragment length polymorphism. The predominant mutations in this population sample are IVS10-11g>a, R261Q, IVS11nt1 and R252W with the frequency 21.7%, 9%, 6.7% and 4.7% respectively. In addition, 6 other mutations have been identified at relatively low frequencies (R261X (4%), 364delG (3.7%), L333F (2%), R408W, R408Q and S67P (0.33%)). These informations provide a good basis for direct DNA diagnosis of PKU in this population.

#### P12.131

#### Case report: 6-year old girl with porencephaly, cataract and microhematuria caused by a *de novo* missense mutation in COL4A1 gene

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Porencephaly is heterogeneous anomaly and usually caused by an ante- or perinatal parenchymal insult in the brain. More recently, the role of mutations in the COL4A1 gene has been shown as a one cause of familial porencephaly. More than 20, mostly familial cases have been previously published. Here we describe an additional case.

This patient was repeatedly investigated due to porencephaly and cataract since birth. Symptomatic focal epilepsy, microcephaly (-3SD) and unspecified microhematuria were additionally noticed. Congenital cytomegalovirus infection was firstly diagnosed, but could not be confirmed by DNA analysis on the newborn screening card. Brain MRI investigation showed bilateral porencephaly with a dark rim in the border of these areas indicating preceding hemorrhage; in other brain areas dark spots were seen, suggestive of hemosiderin deposition and damaged basal ganglia. Second MRI investigation showed additionally a signal abnormality in the hilus of the dentate nucleus. All those findings were suggestive to a mutation in COL4A1 gene.

The COL4A1 gene was analyzed by sequencing analysis and a *de novo* missense mutation was found: c.3707G>A (p.Gly1236Glu). This mutation is likely pathogenic as it is a mutation that changes a highly conserved Gly residue within Gly-Xaa-Yaa repeats in the triple helix domain. Changes in this conserved element are likely to impair the triple helix formation during collagen assembly. It is shown that COL4A1 mutation carriers have great diversity in the clinical expression within the same family. Our described case is showing that the diagnosis should also be considered in sporadic cases of porencephaly.

#### P12.132

#### Recessive primary congenital lymphoedema caused by a VEGFR3 mutation

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The aetiology for primary congenital lymphoedema is not well known. Heterozygous mutations in VEGFR3 have been identified in some familial cases with dominant inheritance, known as Nonne-Milroy disease. Recessive cases of primary lymphoedema with a genetic cause are not known, except for two families with syndromic hypotrichosis-lymphoedema-telangiectasia, with a SOX18 mutation. In this study, we present the first case of isolated primary congenital lymphoedema with

recessive inheritance, caused by a homozygous mutation in VEGFR3. The novel mutation is a transition from alanine-to-threonine in amino acid 855, located in the ATP binding domain of the VEGFR3 receptor. Assessment of receptor function showed impaired ligand-induced internalization and ERK1/2 activity. Moreover, receptor phosphorylation was reduced, although, less so than for a kinase-dead VEGFR3 mutation, which causes Nonne-Milroy disease. In conclusion, a hypomorphic VEGFR3 mutation, with moderate effect on the receptor, in a homozygous state can result in insufficient lymphatic functioning. Thus, in addition to Nonne-Milroy disease with dominant inheritance, VEGFR3 alterations can cause isolated recessive primary congenital lymphoedema. These data expand our understanding of the aetiology of congenital lymphoedema and suggest that large scale screening of VEGFR3 in all primary lymphoedema patients is necessary.

#### P12.133

##### Novel PCCA gene rearrangements causing propionic acidemia represent 21% of the total mutant alleles

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Propionic acidemia is caused by mutations in the PCCA or PCCB genes coding for the two subunits of the propionylCoA carboxylase enzyme. Most of the mutations detected to date in both genes are missense. In the case of PCCA deficient patients, a high number of alleles were uncharacterised, some of them suspected to carry an exonic deletion. We have now employed multiplex ligation probe amplification (MLPA) to screen for genomic rearrangements in the PCCA gene in 20 patients with incomplete genotype. Eight different deletions were found, corresponding to a frequency of 21.3% of the overall PCCA alleles. Two of the exonic deletions were frequent, one involving exons 3-4 and another exon 23. Long-range PCR and chromosomal walking were performed to identify the deletion breakpoints. This revealed two different genomic deletions, both including exons 3 and 4, present in our sample. In all the cases studied, repetitive elements, Alu sequences or simple repeats were found at both sides of the deletion suggesting that these repeats are involved in its generation. The pathogenicity of all the deletions is established based on their predicted effects or on expression analysis of the deletion alleles. This work describes for the first time the high frequency of large genomic deletions in the PCCA gene, which could be due to the characteristics of the PCCA gene structure and its abundance in intronic repetitive elements. Our data underscore the need of using gene dosage analysis to complement routine genetic analysis in PCCA patients.

#### P12.134

##### The effect of lysinuric protein intolerance (LPI) -causing mutations on y+LAT-1 / 4F2hc dimerization analyzed with acceptor photobleaching FRET microscopy

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y+LAT-1 and 4F2hc form a transporter complex for cationic amino acids in the basolateral membrane of epithelial cells, mainly in the small intestine and proximal kidney tubules. Mutations of y+LAT-1, 51 of which are currently known, cause lysinuric protein intolerance (LPI, OMIM #222700), characterized by diminished intestinal absorption of the cationic amino acids lysine, arginine and ornithine and severe loss of these amino acids into the urine. We previously established fluorescence resonance energy transfer (FRET) microscopy as a tool in studying the interactions of y+LAT-1 and 4F2hc. We now have expanded the field into exploring the effects of three different LPI-causing mutations (Finnish founder mutation 1181-2 A->T, G54V and 1548delC) on the dimerization of the transporter complex. Based on the results of this study, the mutations studied do not alter the dimer formation but mutated y+LAT-1 interacts with 4F2hc in a manner similar to that of the wild type. This can be observed by means of fluorescence resonance energy transfer confocal microscopy. The unquenching of the donor fluorescence reports of the occurrence of FRET and thus of the interaction of y+LAT-1 and 4F2hc. Consequently, we conclude that the

interaction between the subunits is a primary phenomenon, which occurs irrespective of the LPI-causing mutations in the light subunit.

#### P12.135

##### A Proteus syndrome case with PTEN gene mutation

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Proteus syndrome is severe disorder of symmetric and disproportionate overgrowth of body parts. The first manifestations of disease are observed at birth and progress significantly with age. Proteus disease belongs to hamartomatous syndromes and is characterized by multifocal overgrowth of tissue, especially ectodermal and mesodermal tissue. We present a case of 12-year-old boy treated in A. Jurasz University Hospital in Bydgoszcz, Poland. Patient was born by natural childbirth at 33 week of gestation. Birth weight was 3200 g. The Apgar scale was scored on 9 points. The family history was negative. Since birth time it has been observed overgrowth and deformation of lower right limb and left foot. Follow-up examination showed extensive dorsal angioma. Based on these findings at 36 months of age the Klippel-Trenaunay syndrome was diagnosed. Because of progression of skin, vascular and soft tissue changes the diagnosis was changed for Proteus syndrome. At the moment patient fulfills diagnostic criteria of Proteus syndrome. Our patient presents main signs of Proteus syndrome like asymmetric overgrowth lower limbs, macrodactyly, protuberant foot, scoliosis, skin lesions and vascular malformation. Progression of soft tissue hyperplasia in right limb and foot deformity is watched

The entire coding sequence of PTEN gene was sequenced by direct PCR product sequencing. The deletion of one nucleotide was identified at the end of intron 7. The mutation was IVS7-3delT. The deletion occurred at splice junction.

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#### P12.136

##### Multiplex ligation-dependent probe amplification refines molecular diagnosis in pseudoxanthoma elasticum

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Background: Pseudoxanthoma elasticum (PXE) is a recessive disorder characterized by oculocutaneous and cardiovascular manifestations, due to mineralization of elastic fibres. The disorder is caused by mutations in the ABCC6 gene, encoding an ATP-dependent transmembrane transporter. By conventional methods, more than 200 missense and nonsense mutations have been described, and the current ABCC6 mutation detection rate accounts for 94% of disease alleles in our cohort. Remaining disease alleles can be partly explained by middle-sized deletions, which can be missed by direct sequencing. We aimed to optimize ABCC6 analysis by screening for such deletions using multiplex ligation-dependent probe amplification (MLPA).

Methods: We performed MLPA in a cohort of 35 out of 331 biopsy proven PXE patients, in whom only one or no mutations were detected after screening for the frequent multi-exon 23-29 deletion and direct sequencing of the ABCC6 coding region and exon/intron boundaries.

Results: Ten deletions were observed comprising five different multi-exon deletions and five novel single-exon deletions.

Conclusion: For this cohort, we demonstrated that approximately 25% (10/41) of currently undetected ABCC6 disease alleles can be identified using MLPA, yielding mutation detection rates of over 95% in our PXE population. Our results demonstrate that the majority of PXE mutations are situated in the ABCC6 gene and indicate that the involvement of another gene locus in classic PXE is unlikely. These results improve the efficacy of familial screening and genetic counseling in PXE. Therefore, we propose MLPA as a valuable additional screening method in the molecular analysis of the ABCC6 gene.

## P12.137

## Detection of large rearrangements by MLPA in severe hemophilia patients

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Determination of genomic rearrangement responsible of disease was a challenge in the past requiring time and critical standardization techniques. The recent arrival of MLPA gene dosage kit made it possible to detect these rearrangements quickly and easily in a single reaction.

Large deletions encompassing from one exon to the complete Factor 8 (*F8*) gene account for about 5% of the causal mutations of severe X-linked hemophilia A leading to an absence of factor VIII. MLPA analysis was performed in 38 DNA unrelated Belgium patients with severe hemophilia A after a negative genetic testing for intron 22 and 1 inversions. DNA of 3 unrelated symptomatic female carriers presenting with factor VIII deficiency in the absence of a family history or in absence of paternal investigation were also included.

MLPA assay identified 3 large deletions (exon 5, exon 15 and exons 23 to 26) and 2 large duplications (exon 1 and exons 1 to 22). For carriers without familial contribution, a decreased copy number compared to control DNAs of exon 1 to 12 in patient 1, of exon 2 in patient 2 and of exon 26 extending to the 3'UTR region in the third patient was identified.

In conclusion, MLPA technology provides an efficient test to search large genomic rearrangement in patients with severe hemophilia A. If deletions are common, a very few cases with duplication was reported. This technique is also warranted in female patients presenting with "de novo" factor VIII deficiency or without familial investigation predicting the severity of the disease.

## P12.138

## Renal hereditary tubulopathies: twelve years experience of genetic analysis

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Bartter (BS) and Gitelman (GS) syndromes are rare autosomal recessive tubular disorders characterised by hypokalemic metabolic alkalosis. So far five genes responsible for the disorders have been identified.

The aim of the study was to characterise and make observation about the molecular defects and the phenotype of the patients clinically diagnosed as BS/GS.

Genotyping was performed on 237 patients by sequence analysis of 4 out of 5 genes: the sodium-chloride cotransporter (*SLC12A3*), the sodium-potassium-chloride cotransporter (*SLC12A1*), the inwardly-rectifying potassium channel (*KCNJ1*) and the chloride channel (*CLCNKB*) genes. For each patient the most probable candidate gene was selected on the basis of laboratory findings and clinical features.

One hundred forty-four patients (61%) showed two mutations: 19 patients resulted as BS type I, 13 as BS type II, 23 as BS type III and 89 as GS. Thirty patients (13%) were simple heterozygotes.

A recurrent mutation was observed on GS patients coming from northern Italy. The evidence of peculiar mutations may facilitate the molecular diagnosis. Among the tubulopathies, GS is confirmed to be the predominant genetic form and the syndrome with the more frequent unknown alleles (20%).

All antenatal BS type II patients had a complete molecular characterisation, with all the mutations clustered in exon 5.

BS type III patients revealed more peculiarities: the entire gene deletion was more frequent among Sardinian probands whereas a chimaeric gene (*CLCNKA-CLCNKB* fusion gene) was mainly detected in Puglia. Moreover, BSIII presented a phenotypic overlapping more frequent with GS then with antenatal BS.

## P12.139

## Identification of allelic expression differences (eQTLs) in retinal expressed (disease) genes

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Reduced penetrance and variability in disease expression with respect to onset, course and severity is common in retinal dystrophies and can be observed even between and within families with the same primary gene defect. It has been suggested that modifications in gene regulation are responsible for much of the observed phenotypic variations. Expression quantitative trait loci (eQTL) studies have become a widely used tool for identifying genetic variants that affect gene regulation. For identifying such eQTLs we crossed five inbred mice (C57Bl/6, Balb/c, CAST, CBA and LP) to form a heterozygous but identical F1 generation and isolated DNA and RNA from ear and retina, respectively. Then, we screened 20 different retinal disease genes for heterozygous cSNPs applying PCR and sequencing. To determine allelic expression differences based on the identified cSNPs, we applied Pyrosequencing assays on RT-PCR amplified retinal cDNAs. The results were calibrated for equimolar ratios by used genomic DNA as a control.

Using the Pyrosequencing technology, a highly accurate method to detect allele-specific expression differences, we have seen in 4 different genes an allelic imbalance. In one of those genes we can see the allelic imbalance already on the genomic level suggesting a copy number variation. In another gene, we were able to detect a mutation within this gene that leads to a premature termination codon leading to downregulation of the mutant transcript due to the nonsense mediated decay. For the other two genes we will determine the promoter sequences and identify variants functionally assessed applying reporter gene assays.

## P12.140

Screening of the *CERKL* c.238+1G>A mutation in 124 Spanish families affected by Autosomal Recessive Retinitis PigmentosaA. Avila-Fernandez<sup>1,2</sup>, E. Vallespin<sup>1,2</sup>, D. Cantalapiedra<sup>1,2</sup>, M. Garcia-Hoyos<sup>1,2</sup>, R. Riveiro-Alvarez<sup>1,2</sup>, J. Aguirre-Lamban<sup>1,2</sup>, A. Gimenez<sup>1,2</sup>, M. J. Trujillo-Tiebas<sup>1,2</sup>, C. Ayuso<sup>1,2</sup>;<sup>1</sup>Fundacion Jimenez Diaz, Madrid, Spain, <sup>2</sup>CIBERER, Madrid, Spain.

Purpose: Retinitis pigmentosa (RP) is a genetically heterogeneous group of inherited retinopathies. Up to now, mutations in 21 genes have been reported to cause the autosomal recessive retinitis pigmentosa (arRP). Three mutations in the *CERKL* gene have been found to cause arRP in different populations. The purpose of the present study was to determine the prevalence of the c.238+1G>A *CERKL* mutation in Spanish patients affected by arRP.

Patients and Methods: To test 124 families affected by arRP for the *CERKL* c.238+1G>A mutation, exon 1 was studied by automated DNA sequencing.

Results: No mutated allele was found in our affected population, in contrast to what has been described in the Yemenite Jewish population.

Conclusions: The *CERKL* c.238+1G>A mutation seems to be an unique mutation to the Yemenite Jewish population, being the *CERKL* p.Arg257ter mutation an exclusive cause of arRP in the Spanish population and the second most frequent mutation in Spanish cases. Therefore, the Spanish population affected by arRP presents different frequencies for different mutations in the *CERKL* gene, when it is compared with other populations.

## P12.141

## A single-base substitution within an intronic repetitive element causes dominant retinitis pigmentosa with reduced penetrance

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We report the study of a previously described family with an autosomal dominant form of retinitis pigmentosa previously linked to the *PRPF31* gene, causing hereditary blindness with reduced penetrance. Sequencing of all exons and introns of *PRPF31* by both the classical Sanger and ultra-high throughput (UHT) techniques, resulted in the

identification of the c.1374+654C>G variant, which was located deep within intron 13. *In silico* analyses suggested that this mutation leads to the creation of a strong donor splice site. Analyses of mRNA derived from patient cell lines indicated that 2 mutant isoforms, both containing parts of intron 13, are synthesized from the *PRPF31* allele carrying this mutation. These mRNAs harbour premature termination codons, and were shown to be present in reduced levels in patient cell lines due to their degradation by the nonsense-mediated mRNA decay. Protein analysis revealed a decrease in the amount of full length *PRPF31* protein and the lack of mutant proteins in patient cell lines. Our results indicate that this mutation is pathogenic and, as with the vast majority of *PRPF31* mutations described so far, leads to the reduction of functional *PRPF31* protein and, consequently, that haploinsufficiency is the cause of retinitis pigmentosa in the studied family.

#### P12.142

##### Novel mutation in RP2 gene in Russian family with X-linked retinitis pigmentosa type 2

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X-linked retinitis pigmentosa type 2 (RP2) - is a severe form of congenital X-linked retinal degeneration, characterized by constriction of the visual fields, night blindness and fundus changes, including 'bone corpuscle' lumps of pigment with a severely reduced visual acuity outcome.

*RP2* gene, responsible for X-linked retinitis pigmentosa type 2, is located on Xp11.3, consist of five exons and encodes a protein of the same name RP2 which links the cell membrane with the cytoskeleton in photoreceptors of the eye.

The purpose of our study was searching for a disease-causing mutation in Russian family with RP2. The penetrance among female carriers is incomplete in the family. Among 28 persons of a four-generation family, in which 6 individuals were affected (5 males and a female), 26 individuals, including 6 affected were involved in DNA-study. Genotyping analysis with polymorphic microsatellite markers NDPCA, DXS1055 and DXS1003 from Xp11.3 showed the evidence of linkage the disease with RP2 locus on chromosome X. Sequencing analysis of all exons and intron-exon junctions of *RP2* in affected man revealed a novel mutation- small deletion c.10-12delTTC in exon 1 of *RP2*. The mutation c.10-12delTTC was identified in all affected men in hemizygous and in all women-carriers in heterozygous state in the family.

The results of the DNA-study revealed a novel disease-causing mutation c.10-12delTTC in *RP2* gene, leading to X-linked retinitis pigmentosa type 2.

#### P12.143

##### Expression and siRNA interference of Rhodopsin *cis*-acting splicing mutants associated with autosomal dominant Retinitis Pigmentosa

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Retinitis Pigmentosa (RP), a clinically and genetically heterogeneous group of retinal degeneration disorders affecting the photoreceptor cells, is one of the leading causes of genetic blindness. Mutation in the rhodopsin gene (RHO) is the most prevalent cause of adRP (autosomal dominant RP).

Two *cis*-acting mutations (c.531-2A>G and c.937-1G>T), that lead to a deficient pre-mRNA splicing, affect the splice sites of RHO and are linked to adRP while a similar *cis*-acting mutation (c.936+1G>T) has been linked to autosomal recessive RP (arRP). Transcriptional expression analysis shows that *cis*-acting splicing mutations causing adRP use intronic and/or exonic alternative splice sites while arRP mutation results in a total exclusion of exon 4. Although protein expression analysis confirms the translation of three RHO mutants, if some of these mutants (carrying a premature termination codon) are targeted by a NMD mechanism is being studied.

Since most mutations causing adRP have a dominant-negative effect, three siRNA molecules have been designed to interfere the mutant transcripts detected in adRP families. Two of them specifically eliminate the desired product.

#### P12.144

##### Identification of mutations in the intracellular Ca<sup>2+</sup> release channels caused cardiac and skeletal muscle disorders

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Rapid mobilization of calcium from the sarcoplasmic reticulum (SR) into cytosol triggers activation of contractile elements, and it is therefore a fundamental process in the physiology of heart and muscles. The channels that regulate the duration and amplitude of calcium efflux from the SR are the ryanodine receptors (RyRs). Three subtypes of these proteins exist: RyR1 is mainly expressed in skeletal muscle, RyR2 is highly represented in cardiac tissue, and RyR3 is preferentially expressed in the brain. Mutations of RyR1 muscle isoform have been associated with predisposition to 2 diseases, malignant hyperthermia and central core disease. Mutations in the RyR2 gene have been identified in families and in sporadic patients affected by catecholaminergic polymorphic ventricular tachycardia (CPVT) and arrhythmogenic right ventricular dysplasia type 2 (ARVD2). The distribution of mutations is identical for RyR1 and RyR2. Most RyRs mutations are clustered in the amino terminus, in the FKBP12.6-binding domains, and in the transmembrane domains of the proteins. We performed RYRs mutation screening based on clustering of known mutations along the RyR1 and RyR2 genes. A multi-step approach is proposed: melting curve and HRM analysis of critical exons, DNA and cDNA sequencing of MH critical regions. We are able to identify pathogenic mutations in the clinically affected proband. DNA analysis can be extended to all family members to identify those are asymptomatic but genetically affected. Detection of RyRs mutations is important because a preventing of fatal cardiac and skeletal muscle disorders in genetically affected patients has been shown to be effective.

#### P12.145

##### Two heterozygous ITPR1 deletions in German families with dominant ataxia

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At least 28 loci have been linked to autosomal dominant spinocerebellar ataxia (ADCA). Causative genes have been cloned for nine nucleotide repeat expansions (SCA1,2,3,6,7,8,10,12&17) and eight genes with missense mutations (SCA4,5,11,13,14,15(16),27&28). Recently, heterozygous genomic deletion comprising the ITPR1 gene on human chromosome 3p24 have been identified as the molecular defect underlying SCA15 in Australian and Japanese ataxia families. In order to assess the prevalence and clinical phenotypes of SCA15, we screened 69 patients with autosomal-dominant ataxias for genomic deletions in exons 1 and 4 of the ITPR1-gene.

Two index patients showed relative gene dosage reduction for both exons after qPCR indicating a heterozygous genomic deletion for at least exon 1 and exon 4 of the ITPR1-gene. To validate these findings we performed a high density SNP genotyping array (Affymetrix 6.0). Copy number analysis validated both heterozygous genomic deletions deleting approximativly 200kb and 500kb, respectively. Both patients had phenotypes compatible with rather pure cerebellar ataxia.

In our entire ADCA cohort (n=274), SCA15 is a rare cause of spinocerebellar ataxia in Caucasians accounting for approximatively 1% of dominant ataxias. Noteworthy this prevalence is comparable to SCA14 and higher than SCA11 and SCA27.

#### P12.146

##### SCA15/16: Phenotype in 4 families with deletions of the ITPR-1 gene

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Spinocerebellar ataxia type 15 (SCA15/16) is a recently identified dominant ataxia caused by mutations in type 1 inositol 1,4,5-triphosphate receptor (ITPR1). Molecular analysis used micro array to detect rearrangements as described before (van de Leemput, PLoS Genet 2007) in 76 index cases with autosomal dominant cerebellar ataxia excluded from polyglutamine expansions. Four index cases (5%) carried a heterozygous ITPR1 deletion; exact size of the deletions has not been yet determined.

There were 12 patients in 4 families with a mean age at onset of  $35 \pm 15.8$  years (n=11; range 18-66); mean disease duration of  $15.8 \pm 13$  years (n=11; range 1-43). The first symptom was cerebellar gait ataxia in 10/11; one patient presented with writing difficulties and hand tremor. All patients developed progressive gait and limb ataxia, with dysarthria in 9 cases. Disease progression was slow in most, none was wheelchair bound. Ocular abnormalities included the presence of nystagmus (n=9), saccadic pursuit (n=5) and intermittent diplopia (n=4). Only one patient had pyramidal signs. Extrapyramidal signs, cognitive decline or sensory abnormalities were absent. Cerebral MRI revealed vermic atrophy in six patients and global cerebellar atrophy in three.

In conclusion SCA 15/16 is rare among French patients with dominant cerebellar ataxia. The overall phenotype is pure cerebellar ataxia with slow disease progression.

#### P12.147

#### *SHH* mutations are an uncommon cause of developmental eye anomalies

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Sonic hedgehog (*SHH*) gene encodes a key morphogen implicated in patterning of the ventral neural tube, the anterior-posterior limb axis and the ventral somites. Mutations in *SHH* are a cause of holoprosencephaly (HPE), a disorder in which the developing forebrain fails to separate correctly into right and left hemispheres. HPE presents a wide spectrum of clinical severity, ranging from cyclopia, proboscis-like nasal structure and midfacial clefting in most severe cases to microcephaly, mild hypotelorism, and eye and tooth anomalies in the milder ones. Familial cases of HPE are typically inherited in an autosomal dominant fashion with reduced penetrance and variable expressivity. In this study, we screened the gene *SHH* in a cohort of 250 cases with anophthalmia-microphthalmia and coloboma via chromosome analysis, dosage analysis with by multiplex ligation-dependent probe amplification (MLPA), high resolution melting curve analysis and bidirectional DNA sequencing. These efforts collectively led to identification of an interstitial deletion on chromosome 7q36.1-q36.3 including the *SHH* gene in a girl with microphthalmia in the right eye and coloboma of the iris and retina in the left eye, a novel 24bp *SHH* intragenic deletion in a girl with unilateral microphthalmia, and a missense mutation in a boy with unilateral microcornea, microphthalmia and iris coloboma. Our results suggest that the *SHH* mutations are an uncommon cause of AM.

#### P12.148

#### The involvement of PI3K signalling pathway in spinal muscular atrophy risk disease: preliminary molecular data

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Spinal muscular atrophy (SMA) is a genetic condition characterized by motor neuron apoptosis, followed by progressive muscle weakness and in many cases by death. It is well known that the disease determining gene is SMN, but the molecular mechanism is still unclear. The PI3K signalling pathway has been often reported as being responsible for the motoneuronal death and can be involved in SMA onset or evolution.

We proposed to evaluate the involvement of PI3KR1 and IGF1R genes polymorphisms (rs3730089 and rs2229765) in SMA risk disease.

In the study were included 38 SMA patients clinically and molecular diagnosed, after the informed consent was obtained. The PI3KR1 and IGF1R genes polymorphisms genotyping was also assessed for 31 control subjects, without neuromuscular problems. Statistical analyses were therefore performed.

The Hardy-Weinberg equilibrium law was respected for the both polymorphisms. The odd ratios with 95% confidence intervals estimated for the risk genotypes, do not revealed statistically significant results. In the case of PI3KR1 gene, the  $OR_{AA} = 0.81$ , 95% CI:0.0487<OR.  $R.<13.513$ , Yate's correction = 0.56, meanwhile for the IGF1R polymorphism  $OR_{GG} = 0.94$ , 95% CI:0.3495<OR.<2.5578, p=0.91. Regarding the risk conferred by polymorphisms alleles, we could observed an  $OR_A = 1.22$ , p=0.6 in case of PI3KR1 gene and  $OR_G = 1.14$ , p=0.6 for G allele for the other polymorphism.

Our preliminary molecular data do not sustain the hypothesis of the involvement of PI3K signaling pathway in SMA risk disease. A future study with an increased statistical power may clarify this relationship. This research was funded by CNCSIS grant TD 223/2008.

#### P12.149

#### Two unaffected subjects with homozygous deletion of SMN1 gene.

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Spinal muscular atrophy (SMA), an autosomal recessive neuromuscular disease, is caused by homozygous deletion of the SMN1 gene in about 96% of the 5q13-linked SMA patients. SMN1 gene, that mainly produces full-length transcripts, has a highly homologous centromeric copy (SMN2) that produces predominantly exon 7-skipped transcripts. However, there is an inverse correlation between the SMN2 copy number and clinical severity of SMA phenotype. In rare cases, homozygous deletion of SMN1 gene in unaffected siblings of patients with SMA can be observed. Recently, Oprea et al., indicated that very high level of Plastin 3 (PLS3) mRNA, observed in the peripheral blood of unaffected females, could act as a protective gender-specific SMA modifier. In this report, we present two asymptomatic female carriers of biallelic absence of the SMN1 gene. We determined genes organization at the SMA locus by MLPA/Q-PCR approaches that allows the contemporary evaluation of SMN1/SMN2 copy number. In both families we investigated: 1) the copy number of SMN2 gene; 2) the amount of the full-length SMN mRNA; 3) the amount of PLS3 mRNA. The SMN2 copy number resulted the same (4 copies) in the unaffected sister and her affected brother, while 5 copies were detected in the other unaffected female. The amount of full-length SMN mRNA is quite low and comparable between asymptomatic and symptomatic subjects. Finally, the amount of PLS3 mRNA is comparable between all the subjects studied. Altogether these results suggest that other factors than full-length SMN and PLS3 could be important SMA phenotype modifiers.

#### P12.150

#### Molecular Analysis of Exon 7 and 8 of SMN Gene in Turkish Spinal Muscular Atrophy Patients

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Scientific background: The spinal muscular atrophies (SMAs) causing degeneration of the anterior horn cells of the spinal cord characterized by progressive weakness of the lower motor neurons. Several types of SMA have been described depend on age when accompanying clinical features appear. The most common types are acute infantile (SMA type I, or Werdnig-Hoffman disease), chronic infantile (SMA type II), chronic juvenile (SMA type III or Kugelberg-Welander disease), and adult onset (SMA type IV) forms. SMA is diagnosed with detection of homozygous deletions of SMN1 (exon 7 - 8 or exon 7) gene in molecular level.

Objectives: It is aimed to conduct molecular analysis of exon 7 and 8 of SMN gene in sixty five subjects of SMA (66 patients).

Materials and methods: PCR-RFLP method is used for detection of homozygous exon 7 - 8 deletions. PCR-SSCP method was used either to identify for intragenic mutations and especially compound heterozygotes or to confirm some SMA patients homozygous deletions detected by RFLP.

Conclusion: In this study, 94 % (62/66) of SMA patients including all types were found homozygous for exon 7 and 8 deletions with RFLP method. The rate of homozygous deletions determined was 94.7% (18/19) in type I patients, 96% (22/23) in type II and 90% (18/20) in type III. SSCP method was used only for 4 subjects who are clinically diagnosed as SMA patients but not confirmed with RFLP analysis. The results of SSCP analyses led to decision that patients may be of compound heterozygous or intragenic mutations.

#### P12.151

##### New DNA microvariations described by SMRT arrays.

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Introduction: Genome screening using array CGH has great potential in the characterization of unexplained chromosomal aberrations. The whole genome Sub-Megabase Resolution Tiling Array (SMRT array) is capable of identifying microamplifications and microdeletions at a resolution of 100 Kb. Other different techniques, such as MLPA or FISH, are traditionally employed to detect these chromosomal alterations. In this study we show the utility of the SMRT arrays to provide precise information about the size and breakpoints of DNA copy number gains and losses.

Methods: We present a patient with unexplained mental retardation and a male normal karyotype 46, XY. MLPA kit (from MRC-Holland) technique was carried out. A SMRT array, (from Wan Lam Laboratory at the BC Cancer Research Centre) analysis was performed to confirm and describe the alteration.

Results: MLPA study showed a 14q deletion of about 1.5 Mb. The 14q specific probe of MLPA kit was deleted. The SMRT array analysis of the specific 14q32.33 region confirmed this microdeletion and allowed us to exactly describe its size into 2.20 Mb.

Conclusions: The SMRT array study confirms a small deletion of 2.20 Mb unless than 1.5 Mb previously detected by MLPA. SMRT array arises as an effective technique to detect DNA microvariations and provides more information about their size and precise breakpoints.

#### P12.152

##### SNP array analyses can orient molecular diagnosis of autosomal recessive heterogenous diseases in sporadic cases from consanguinuous families

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Molecular diagnosis of rare autosomal recessive diseases with extensive genetic heterogeneity represents a real challenge because

clinical data do not in most cases suggest a particular defective gene. Consanguinity is frequent in such families. Genome wide SNP array analysis allows, by searching for homozygous regions in such patients, the selection of one or few candidate genes in which to search for mutations. We report 8 such cases including 6 sporadic ones, where the disease causing gene and mutation were found using this approach (see table).

Case	Form	Disease	Number of candidate-homozygous segments	Mutated gene
1	Sporadic	Myopathy	1	TRIM32
2	Familial	Spastic paraplegia	1	SPG11
3	Familial	Ataxia	1	AOA1
4	Sporadic	Achromatopsia	2	CNGB3
5	Sporadic	Bardet Biedl	1	BBS1
6	Sporadic	Ataxia	6	FXN
7	Sporadic	Bardet Biedl	2	BBS6
8	Sporadic	Bardet Biedl	2	BBS5

Homozygosity mapping using 50K micro-arrays (Affymetrix) was performed only on the patients and allowed us to identify causative mutation in a significant proportion of sporadic cases affected with different neuromuscular or neurosensory diseases (see table.). This rapid and not too expensive approach is particularly useful for diseases with extensive genetic heterogeneity like Bardet Biedl syndrome (14 genes published to date), limb girdle muscular dystrophy or sporadic ataxias, by selecting only one or two genes for sequencing and identify the private mutation. In some cases, SNP array analysis can reveal consanguinity that was unknown to or denied by the family

#### P12.153

##### Twenty novel mutations in SPG11/spatacsin identified using both direct sequencing and MLPA

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Objective: To extend the SPG11 mutation spectrum and establish the frequency of genomic rearrangements in this gene.

Background: Truncating point mutations in SPG11/spatacsin are the major cause of autosomal recessive spastic paraplegia with thin corpus callosum. Recently genomic rearrangements were also involved.

Methods: 45 unrelated patients with spastic paraplegia with thin corpus callosum +/- mental retardation or cognitive delay were screened using direct sequencing and MLPA.

Results: 25 different SPG11 point mutations, 18 of which were novel, were identified in 16 patients (36%). All mutations but one introduced premature termination codon in the protein sequence and were compatible with a degradation of the corresponding mRNA by the non-sense-mediated mRNA decay. The remaining mutation was a missense variant which alters a highly conserved amino-acid of the protein and was found associated with a truncating mutation. In addition, MLPA analysis detected heterozygous SPG11 micro-rearrangements in two patients who already had a single heterozygous point mutation. Analysis of the affected relatives and parents when possible showed that the mutations segregated with the disease and that heterozygous compound mutations were inherited each from a healthy parent. Only two patients out of 16 had homozygous mutations; the remaining 14 patients had heterozygous compound mutations. Finally, we identified new missense polymorphisms that did not segregate with the disease.

Conclusions: These findings expand the SPG11 mutation spectrum and highlight the importance of screening the whole coding region with both direct sequencing and a quantitative method.

Rare missense polymorphisms are frequent in SPG11, complicating interpretation of diagnosis.

#### P12.154

##### SPG4 mutations can mimic primary progressive multiple sclerosis on clinical, biological and MRI aspects

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The most common form of autosomal dominant hereditary spastic paraplegia (AD-HSP) is caused by mutations in the SPG4/SPAST

gene, encoding spastin. SPG4-HSP and generally described as a pure form of the disease—that is, as spastic paraparesis often associated with a decreased sense of vibration in the lower limbs and urinary problems. However, it is characterized by a large variability in the age at onset (ranging from early infancy up to the eighth decade). Patients usually have a family history of the disease but some may also present as sporadic cases due to incomplete penetrance or censor effects. In the latter, non-genetic causes of spastic paraparesis, such as primary progressive multiple sclerosis, (PPMS) are usually searched for. We report 3 patients with a primary diagnosis of PPMS, based on MRI findings, who carried a SPG4 mutation. Interestingly, 2 out of the 3 patients had a family history of gait disorder or clinical diagnosis of MS and in one, the father died at age 60 (censor effect). The remaining patient fulfilled Mc Donald's criteria (MRI and CSF) for MS. Those findings must lead to important caution with PPMS diagnosis not to underestimate genetic spastic paraparesis because of consequences for appropriate genetic counselling. Those results could also modify our understanding of the mechanisms of demyelinating diseases and of the interactions between axonal degeneration and inflammation.

#### P12.155

#### Molecular analysis of spinal muscular atrophy in Iranian population

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Spinal muscular atrophy (SMA) is an autosomal recessive genetic disorder of motor neurons. Defects in genes for survival motor neuron (SMN) and neural apoptosis inhibitory protein (NAIP) have been shown to be associated with the disease. Among the genetic defects, deletions in exon 7 and 8 of SMN as well as exons 4 and 5 of NAIP gene were found to be most significant. In this study, deletions in SMN and NAIP genes were examined in 35 unrelated SMA patients (14 type I, 5 type II, 16 type III) patients. Deletion frequency in SMN (E7 and 8) and NAIP (E4 and 5) in patients with SMA type I was 92.8%, 42.8%, 64.3%, 78.6%; in type II, 60%, 20%, 80%, 80%; and in type III, 16.6%, 6.25%, 43.7%, 43.7%, respectively. About 7.1% of patients with SMA type I; 20% with type II and 56.2% with type III showed no deletions for the exons examined. Moreover, homozygous deletion in E7 and/or 8 of the SMN gene was found in 62.5% of the patients, with high frequency in both type I (78.5%) and type II (80%), and less frequency (48.5%) in type III. Similarly, homozygous deletions of E4 and/or E5 of NAIP gene was highest in type I (92.9%) compare to type II (60%) and type III (6%). Our data suggest a strong association of deletions in E4 and E5 of NAIP together with deletions in SMN (E7 and 8) with more severe form of SMA (SMA type I and II) in Iranian population.

#### P12.156

#### Estimation of SMN2 copy number in 536 unrelated Spanish SMA patients by Multiplex-Ligation dependent Probe Amplification (MLPA)

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Spinal muscular atrophy (SMA) is a neuromuscular disorder caused by absence or mutations in the SMN1 gene. SMA patients are classified into 3 groups, type I (the most severe), type II (the intermediate form) and type III (the less affected) according to age of onset, achieved motor abilities, and life span. SMN2 is the SMN1 highly homologous copy that is considered as a modifier of the disease.

We estimated the SMN2 copy number in 536 unrelated SMA patients from different Spanish centres. We employed the Multiplex-Ligation dependent Probe Amplification (MLPA) technique, which includes a mixture of specific probes for the SMA locus.

The majority of the type I patients showed two copies of the SMN2 gene (209/261, 80%), type II patients presented mainly 3 SMN2 copies

(126/150, 84%), whereas 91% (114/125) of type III patients have 3 (67%) or 4 (24%) SMN2 copies. These results confirm that SMN2 copies are strongly related to disease severity. However, the correlation is not absolute (i.e. 36 type I patients showed three SMN2 copies and 9 type III patients had two copies). This study performed in a large cohort of subjects, allowed us to improve the genetic characterisation of the SMA locus. Moreover, it will be useful to define a subtype of patients (i.e. those with three SMN2 copies and different SMA type) to further investigate the functional copies of SMN2 and possible modifiers of the phenotype. Supported by GENOME Project, CIBERER and FIS 05-2416.

#### P12.157

#### High throughput, complete genotyping of the TNFRSF1A gene. This project is supported through Coordination Theme 1 (Health) of the European Community's FP7

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The TNF Receptor Associated Periodic Syndrome (TRAPS) is a rare autosomal dominant multisystemic autoinflammatory disorder, caused by sporadic mutations in the TNF super family Receptor 1A gene (*TNFRSF1A*). Genetic diagnosis of TRAPS consists usually of sequencing of exons 2-4 of *TNFRSF1A* gene. However, mutations which cause TRAPS have been also found outside these exons; therefore, a system that would offer complete genotyping of the *TNFRSF1A* gene would be highly advantageous. We are currently developing an answer to this need using a new, high throughput technique, for simultaneous detection of both known and novel point mutations (SNPs and pathogenic mutations) and of large-scale genetic rearrangements in the *TNFRSF1A* gene. This system, called EMMA (enhanced mismatch mutation analysis) is based on electrophoretic heteroduplex analysis (HDA) and semiquantitative multiplexed PCR by multi-capillary electrophoresis. The EMMA (Fluigent, France) mutation detection method is an alternative to dHPLC and sequencing. It combines all the advantages of the screening before sequencing strategy: high throughput, major reduction in sequencing costs and high productivity. The presence of both point mutations and long-range rearrangements has a "signature" that can be associated with quantitative and objective numerical criteria. The analysis of electrophoregrams can be fully automated; a feature that for the rare TRAPS case is less imperative, but will be very useful for high-throughput screening of clinically important genes with more prevalent SNPs and pathogenic allele variants.

#### P12.158

#### New trinucleotide diseases analysis based on HPLC and QF PCR

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Trinucleotide expansions are an important mutational form specifically in neurodegenerative disorders such as HD (Huntington disease), different types of SCA (Spinocerebellar ataxia), SBMA (Spinal and bulbar muscular atrophy) and DM1 (Myotonic dystrophy type 1). Trinucleotide disorders are characterized by an increasing of severity of disease course in the next generation of mutation carriers. The number of repeats may reach over 2000 in severe affected individuals. These larger expansions require specific and time consuming methods for identification, such as fragments lengths analysis by polyacrylamide gel electrophoresis (PAGE) or Southern blotting. Identification and exact determination of alleles on the molecular level is very important for diseases diagnosis and prognosis. The aim of our study was to develop a highly sensitive, automated and economical molecular method for determination and characterization of trinucleotide repeat regions. Here we present the molecular test based on high performance liquid chromatography (HPLC) and quantitative fluorescent (QF) PCR for characterization of trinucleotide repeats. We analyzed 403 samples - clinically diagnosed with different trinucleotide disorders. Our results indicated high accuracy and consistency of the data obtained with HPLC ( $\pm 3$  CAG) compared to classical methods based on PAGE and sequencing. The results obtained using combined QF and Long PCR showed higher precision in comparison to the results obtained by traditional techniques. We conclude that HPLC, QF and Long PCR

can be used as a sensitive and efficient alternative diagnostic method compared to conventional techniques for measuring fragment lengths in trinucleotide diseases.

#### P12.159

#### Characterization of two novel mutations in Tricho-rhino-phalangeal syndrome

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Mutations of the *TRPS1* gene lead to the tricho-rhinophalangeal syndromes (TRPS) types I or III. They are characterized by craniofacial and skeletal abnormalities. Cone-shape epiphyses are the characteristic radiographic findings. The patients have sparse scalp hair, a bulbous tip of the nose, a long flat philtrum, and a thin upper vermillion border. TRPS III is similar to TRPS I except by the presence of severe brachydactyly due to short metacarpals and severe short stature. TRPS II or Langer-Giedion syndrome is a microdeletion syndrome affecting both the *TRPS1* and *EXT1* genes and differs from TRPS I and TRPS III by the presence of mental retardation and multiple cartilaginous exostoses. TRPS I is inherited as an autosomal dominant trait and is due to molecular defects in the *TRPS1* gene. In the present study we describe two novel mutations in 2 families affected with TRPS I. We screened all exons of the *TRPS1* from the patients. The mutation analysis showed missense mutation in exon 3 and nonsense mutation in exon 4 of the *TRPS1* gene. The TRPS I phenotypes of most patients with a nonsense mutation and with a *TRPS1* deletion or disruption are very similar. All nonsense mutations in a heterozygous state, reduce the doses of *TRPS1* protein, supporting that haploinsufficiency is the cause of TRPS. Our data show a higher genotypic spectrum in the TRPS I and demonstrate that mutations within the initial region of the enzyme and in the GATA region of the *TRPS1* gene result in TRPS I syndrome.

#### P12.160

#### Large genomic rearrangements in the Usher genes

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Usher syndrome is an autosomal recessive hearing loss which combines sensorineural deafness and retinitis pigmentosa. It is both clinically and genetically heterogeneous. Three clinical subtypes are defined in respect to the vestibular dysfunction and the degree of hearing loss. Type I (USH1) patients have profound hearing loss (HL) and vestibular dysfunction. Type II (USH2) is the most frequent form and patients have moderate to severe HL and normal vestibular function. Type III (USH3) is characterized by progressive HL. So far, 9 genes are known to be responsible for Usher syndrome (5 for USH1, 3 for USH2 and 1 for USH3). We have developed an exhaustive molecular analysis based on sequencing of the 9 genes (exons + flanking intronic regions). More than 180 USH families have been analysed so far. We identified the pathogenic mutations in most cases; however, 15% of our cohort carries either one or no mutation in any of the genes. The absence of mutation can sign the presence of large rearrangements that remain undetected by sequencing. Therefore we have developed a semi-quantitative non-fluorescent multiplex assay to identify large deletions or duplications. In addition, a MLPA kit has been recently designed for *PCDH15*.

We have detected so far 12 large genomic rearrangements in 11 families. The breakpoints could be identified for 6 families. This study shows that large genomic rearrangements are implicated in at least 6 % of the Usher cases and their screening should be included for efficient molecular diagnosis.

#### P12.161

#### Functional analysis of splicing mutations in Usher syndrome genes

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Usher syndrome (USH) is an autosomal recessive disorder characterized by sensorineural hearing loss, Retinitis Pigmentosa and variable vestibular areflexia. Clinically three subtypes are distinguished (USH1-USH3) and to date, nine genes have been associated to the disease. The most prevalent USH genes are *MYO7A* for USH1 and *USH2A* for USH2, with prevalences that range 29-55% and 75% respectively.

Five sequence variants, suspected to affect the splicing process, had been identified in our cohort of USH patients: c.2283-1G>T and c.5856G>A in the *MYO7A* gene, and c.1841-2A>G, c.2167+5G>A and c.5298+1G>C in the *USH2A* gene.

In the present study, minigenes based on pCI-Neo Mammalian Expression vector were used to investigate the implication of these variants in the mRNA processing. Exons and flanking intronic sequences, both wild type as mutated, were cloned. After transfection of COS-7 cells, RNA was extracted, retrotranscribed to cDNA and analyzed.

All changes were observed to affect the splicing process, being responsible for the skipping of implicated exons. Furthermore, the mutation c.2167+5G>A generated too an alternative splicing, where the final of the affected exon is lost.

It is important to analyze the role of putative splicing variants in USH genes in order to determine their pathologic effect. Most USH genes show an expression profile restricted to hardly accessible tissues so, strategies as minigenes are needed to determine the implication of identified variants in the mRNA processing.

#### P12.162

#### Unclassified variants in Usher syndrome and related disorders

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Genes involved in Usher syndrome, the major cause of hereditary deaf-blindness, are particularly prone to alterations of unknown clinical significance (Unclassified Variants, UVs). In the *MYO7A* and *USH2A* genes, such alterations can account for up to 50% of the newly identified variants in molecular diagnosis. The most encountered type of UV is missense variants, which can affect the protein structure and therefore its function. *In vitro* assessment is there difficult and expensive, and *in silico* studies represent an attractive way to classify these variants. We present here a multiple step analysis which combines biological observations and *in silico* studies. Six items are taken into account (4 of which are discussed in Greenblatt *et al.*, 2008), and are presented below:

Review of published literature	Biological observations		In silico studies		
	Position in cis or trans of a second alteration	Presence or absence in relevant control DNAs	Ortholog conservation	Conservation in similar protein domains	3D analysis

Each item is then rated following precise rules, and the sum allows the classification of missense variants on a four-grade scale, UV1-4, as recommended in the guidelines published by the Clinical Molecular Genetics Society, UV1 corresponding to certainly non-pathogenic variants and UV4, considered as certainly pathogenic variants. This empirical method has already been applied to 115 missense variants identified in 6 genes responsible for Usher syndrome or related non-syndromic affections, and will be extended to all identified missense variants in these genes. Moreover, this method could be applied to variants identified in genes involved in any recessively transmitted Mendelian disease.

#### P12.163

#### Vascular Ehlers-Danlos syndrome in Italy: identification of 15 novel and 2 known *COL3A1* mutations

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Vascular EDS (vEDS) (MIM #130050) is a dominantly inherited disorder, whose clinical diagnosis is made by major and minor criteria (e.g., easy bruising, thin skin, typical *facies*, and fragility of arteries, or internal organs). The first major complication occurs in 25% of vEDS patients by the age of 20, and in 80% by the age of 40; the median survival is 48 years. vEDS is due to mutations in *COL3A1* gene. About 200 *COL3A1* mutations have been reported. Mutations in *TGFBR1* and *TGFBR2* cause the Loeys-Dietz syndrome (LDS) type II (MIM #610380), presenting with vEDS major signs and without cardinal features of originally described LDS.

In this work we report the characterization of Italian vEDS patients. In 17 out of 32 probands, with presumed vEDS, we disclosed 15 novel and 2 known *COL3A1* mutations: 13 (76.5%) missense, and 4 (23.5%) splicing mutations. All the missense mutations affected a glycine in the collagenous domain of the protein, and all the splicing mutations the donor splice site, leading to exon in frame skipping. No *TGFBR1* and *TGFBR2* mutations were detected in *COL3A1* negative patients. The median age of the first complication in 15 out 17 probands was 28.5 years. Two probands (at 35 and 12 years) and 3 relatives died for abdominal aorta rupture. The most involved vessels were the medium-sizes arteries, mostly the splenic and hepatic ones. The majority of the patients were diagnosed after a major event. These data add insights to the knowledge of vEDS genotype-phenotype correlation.

#### P12.164

#### The influence of VEGF polymorphism on the progression of chronic glomerulonephritis

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The role of vascular endothelial growth factor (VEGF), a potent angiogenic agent, in the pathogenesis of different glomerulonephritides (GN) has been studied intensively. We investigated the influence of VEGF polymorphism at position -2578 (A/C) of the VEGF promoter on the progression of two common GN - focal segmental glomerulosclerosis (FSGS) and IgA nephropathy (IGAN).

247 Czech patients with FSGS and IGAN entered into study. Patients were divided into rapid progressors (RP) - 99 pts (64 males, 35 females, mean age 45.4 ± 10.7 years) with renal failure during 5 years since renal biopsy and slow progressors (SP) - 148 pts (91 males, 57 females, mean age 46.5±12.1 years) with stable renal function. 100 genetically unrelated healthy subjects were control group (CG). Genomic DNA was amplified by PCR with published primers. A  $\chi^2$ -test was used to compare the distribution of genotypes according to recessive and dominant genetic model between RP and SP.

The distribution of the -2578 VEGF polymorphism:

		AA (%)	AC (%)	CC (%)
FSGS	RP	27.3	49.1	23.6
	SP	23.1	50	26.9
IGAN	RP	22.7	40.9	36.4
	SP	22.1	52.5	25.4
CG		21	54	25

There was a tendency to negative prognostic value of the C allele on IGAN, however a significant influence of VEGF-2578 polymorphism on the progression of FSGS and IGAN was excluded.

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#### P12.165

#### Williams-Beuren Syndrome in a Bulgarian patient diagnosed by MLPA kit for microdeletion syndromes.

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William-Beuren syndrome (WBS) syndrome is an autosomal dominant disorder clinically characterized by supravalvular aortic stenosis (SVAS), multiple peripheral pulmonary arterial stenoses, elfin face, mental and statural deficiency, specific dental malformation, and infantile hypercalcemia.

The genetic cause of WBS is a contiguous gene deletion of several genes on chromosome 7q11.23. The severity of clinical symptoms and particularly mental retardation may be related to the size of the deletion (the number of genes involved). Recently developed for research purposes Multiplex Ligation-dependant Probe Amplification (MLPA) method provides a possibility to detect copy number changes (deletions and duplications) along a single or a number of genes. We successfully applied MLPA probe mix P245-A1 Microdeletion syndromes to genetically diagnose a patient with clinically suspected WBS. The obtained results showed undoubtedly a reduction of gene dosage for the 3 specific WBS probes: two within Elastin(ELN) gene and one in (LIMK1) gene, both localized in 7q11.23. The detected deletion in our patient was associated with multiple peripheral pulmonary stenoses, supravalvular aortic stenosis, moderate mental retardation, cognitive problems associated with attention deficiency and hyperactivity. Facial dysmorphology was also present: thick lips, macrorhachidism, micrognathia.

The MLPA analysis proved to be useful in routine genetic diagnosis of microdeletion syndromes and particularly William-Beuren syndrome.

#### P12.166

#### Is TRIM50, a Williams Beuren syndrome gene, at the intersection of autophagy and proteasome pathways?

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Proteasome and Autophagy catabolic pathways have been implicated in many human disorders. Recently, we showed that TRIM50 acts as an E3-ubiquitin ligase. TRIM50 is hemizygous in the Williams Beuren syndrome (WBS), a contiguous genetic disorder, caused by a 1.5 Mb deletion at 7q11.23 that include about 25 genes. Although some of the WBS phenotypes have been associated to some of the deleted genes, the contributions of the remaining genes, including TRIM50, to the multiple defects are still undetermined. To get insight on its role and to identify putative TRIM50-interacting peptides we performed fluorescence and electronic microscopy and mass spectrometry.

We founded that TRIM50 protein localizes in highly mobile, labile and dynamic cytoplasmic bodies. CLEM-microscopy showed that it localizes in the multi-vesicular structures similar to the autophagosome and notably it colocalizes with LC3, a specific marker of autophagosomes. Consistently, nano LC-MS/MS identified a number of putative TRIM50-interacting proteins known being implicated in the proteasome and autophagy. Among them we showed that TRIM50 interacts with P62/SQSTM1, an essential protein involved in the autophagic flux. p62 is able to transfer misfolded and ubiquitinylated proteins to the autophagosomes for degradation and as TRIM50 is an E3-ubiquitin ligase, we can speculate that TRIM50 ubiquitinates its substrates that are, then, shuttled to the autophagosome for degradation via TRIM50-p62 complex.

These results show an unexpected role for TRIM50 on protein degradation pathways. We anticipated that the haploinsufficiency of TRIM50 could account for a consistent part of WBS phenotype through the accumulation and/or abnormal degradation of TRIM50 substrates.

**P12.167****Pattern of direct molecular diagnosis of Wilson disease in Moldavian patients****N. Mocanu, V. Sacara;***National Center of Reproductive Health and Medical Genetics, Chisinau, Moldova, Republic of.*

Wilson's disease (WD) is a rare inborn error of metabolism caused by a defect in ATP7B, a protein necessary for proper copper excretion into bile. We investigated Moldavian families with WD for identification of mutations of the ATP7B gene in 14 and 15 exons using single strand conformation polymorphism method (SSCP) of DNA molecule.

We analyzed 34 Moldavian patients with WD. The diagnosis was established in any patients with unexplained liver disease along with neurological or neuropsychiatric disorder, presenting of Kayser-Fleischer rings, low serum ceruloplasmin level, the amount of copper excreted in the urine in a 24-hour period. Our patients had presented with predominantly hepatic, neurological or psychiatric manifestations. In exon 14 ATP7B gene have been determined missens-mutation His1069Glu in 8 (25, 5%) causes. In 2 (25%) patients the mutation was detected in homozygote form, but in 6 (75%) patients in heterozygote form. In the 15 exon of the ATP7B gene we don't detected the fragments with abnormal electroforetic activity. The search of deletion in the 15 exon (C3400delCgene) in studied patients hadn't any results.

The detected of missens-mutations in 23,5 % of causes has a major importance for diagnosis in Moldavian patients. Frequency of His-1069Glu mutation in Moldavian's patients is lower than in European's patients. The presences in patients with certain clinical manifestation the heterozygous occurrence of missense-mutation can take part a compound phenomenon.

**P12.168****Wolfram syndrome. Clinical and genetic study in an Italian family.****L. Rigoli, G. Salzano, C. Di Bella, M. Amorini, V. Procopio, G. Lo Giudice, P. Romeo, F. Pugliatti, L. Grasso, C. Salpietro, F. Lombardo;***Department of Pediatrics-Policlinico Universitario, Messina, Italy.*

**Background:** Wolfram syndrome (WS) is a recessively inherited mendelian form of diabetes insipidus, diabetes mellitus, optic atrophy, and deafness. Affected individuals may also show renal tract abnormalities as well as multiple neurological and psychiatric symptoms. The causative gene for WS (*WFS1*) encoding wolframin maps to chromosome 4p.16.1 and consists of eight exons, spanning 33.44 Kb of genomic DNA. The current study aimed to contribute to our understanding of the molecular basis of WS in an affected Italian family.

**Methods:** The proband was a 15-year-old girl who exhibited diabetes mellitus at the age of 8 years, diabetes insipidus and optic atrophy at the age of 14 years. No other clinical and instrumental signs were found at the time of our study. Her sister, a 6-year-old girl, was affected by diabetes mellitus (age of onset at 5 years). No other clinical and instrumental signs were detected. The parents were not consanguineous. Blood samples were obtained from the patients and their parents. *WFS1* analysis included exons 1-8 of the coding regions. Results were compared with the *WFS1* genomic sequence (NT-006051.17).

**Results:** The two sisters exhibited a deletion c.1362-1377del16 located at the exon 8 in homozygosity. The same deletion was found in their parents in heterozygosity.

**Conclusions:** Our data underline that the identification of *WFS1* mutations in families with Wolfram syndrome enables specific carrier detection, prenatal diagnosis and delineation of genotype/phenotype correlation.

**P12.169****Four SH2D1A mutations on 7 chromosomes detected in Russian patients with X-linked lymphoproliferative syndrome (XLP)****N. Poltavets<sup>1</sup>, M. Maschan<sup>2</sup>, I. Semyagina<sup>1</sup>, A. Polyakov<sup>1</sup>, I. Kondratenko<sup>2</sup>, A. Maschan<sup>2</sup>, G. Novichkova<sup>2</sup>;**<sup>1</sup>*Research Center for Medical Genetics, Moscow, Russian Federation, <sup>2</sup>Federal Research Clinical Center for pediatric hematology, oncology and immunology, Moscow, Russian Federation.*

XLP is a congenital immunodeficiency disease characterized by defective immune response against Epstein-Barr virus (EBV). The most frequent XLP phenotype is fulminant infectious mononucleosis which is clinically identical to hemophagocytic lymphohistiocytosis. Mutations

in *SH2D1A* are the most frequent cause of XLP. This gene encodes a small intracytoplasmic protein, defects of which cause selective alteration of NK and T cell functions, resulting in an inability to control EBV-infected B cells. Another XLP gene is *XIAP* (or *BIRC4*) encoding the X-linked inhibitor of apoptosis protein.

We have investigated the group of 26 unrelated male patients: 22 with initial clinical XLP diagnosis; 4 with initial clinical FHL (Familial hemophagocytic lymphohistiocytosis) diagnosis without mutations in FHL genes (*PRF1*, *UNC13D*, *STX11*). DNA samples have been investigated for mutation in *SH2D1A* and *XIAP* genes coding areas by direct sequencing.

The analysis of *SH2D1A* coding region revealed 4 different mutations on 7 chromosomes - hole gene deletion on 1 chromosome, c.164G>T (p.55Arg>Leu) CD014961 on 2 chromosomes, c.53insA on 1 chromosome and c.163C>T (p.55Arg>Stop) CD981809 on 3 chromosomes. The c.53insA mutation was new, and others were previously reported. The investigation of *XIAP* coding region showed 3 previously reported polymorphisms (c.1268A>C (p.423Gln>Pro) rs5956583 on 7 chromosomes, c.\*+80G>C rs12838858 on 1 chromosomes and c.\*+12A>G rs28382740 on 4 chromosomes). New nucleotide substitution (c.1056+19A>T) detected on 1 chromosomes wasn't found among previously reported SNP.

According to our data *SH2D1A* gene mutations are the cause of XLP for 27% of the examined cases. No mutations were found in coding region of *XIAP* gene.

**P13. Metabolic disorders****P13.01****Adiponectin gene polymorphism (G276T) in patients with abdominal obesity****V. B. Timoshin<sup>1</sup>, O. D. Belyaeva<sup>2</sup>, E. A. Bagenova<sup>2</sup>, A. V. Berezina<sup>2</sup>, O. O. Bolshakova<sup>2</sup>, E. A. Chubenko<sup>2</sup>, A. E. Garanina<sup>2</sup>, E. I. Baranova<sup>2</sup>, O. A. Bercovitch<sup>3</sup>, V. I. Larionova<sup>1</sup>;**<sup>1</sup>*St. Petersburg State Pediatric Medical Academy, St. Petersburg, Russian Federation*<sup>2</sup>*St. Petersburg State Medical University after Pavlov IP, St. Petersburg, Russian Federation*, <sup>3</sup>*St. Petersburg State Medical University after Pavlov PI, St. Petersburg, Russian Federation*.

The adiponectin gene is a candidate gene responsible for insulin resistance and type 2 diabetes. OBJECTIVE: (1) to determine the frequency of single nucleotide polymorphism 276 G>T of the adiponectin gene in patients with abdominal obesity, and (2) to study an effect of particular genotypes on serum adiponectin level and on insulin sensitivity. STUDY POPULATION: 78 males and 287 females with abdominal obesity aged of 30-55 years. Control group included 119 children and adolescent from St. Petersburg. METHODS: Serum adiponectin, indicators of lipid and carbohydrate metabolism, blood glucose level, waist circle, and body mass index (BMI) were evaluated according to standard protocols. Genotyping was performed by PCR-RFLP. RESULTS: In patients with abdominal obesity, frequency of G-allele of T-allele was 0.68 and 0.32, respectively. There was no difference in GT, GG, and TT genotype distribution and in rates of G- and T-alleles of adiponectin gene between the studied groups. No difference was detected in adiponectin, glucose level, lipid spectrum of the serum, waist circle, and BMI between patients carrying different adiponectin gene genotypes. G-allele carriers (GG 4.65±0.54 and GT 5.68±0.77, p<0.05) had higher HOMA-IR index compared to carriers of homozygous T-allele (2.7±0.4, p<0.05). Insulin level was higher in patients carrying G-allele (22.46±1.46 vs 19.41±1.11, p<0.05) compared to patients carrying TT-genotype (17.11±2.3, p<0.05). CONCLUSION: We did not reveal any differences in adiponectin level in patients carrying various adiponectin gene genotypes. HOMA-IR index and insulin level were higher in patients carrying adiponectin gene G-allele.

**P13.02****Variety of POLG phenotypes and molecular findings in a series of 9 Russian patients****P. Tsygankova<sup>1</sup>, S. Mikhaylova<sup>2</sup>, E. Zakharova<sup>1</sup>, L. M. Kolpakchi<sup>2</sup>;**<sup>1</sup>*Research Center for Medical Genetics, Moscow, Russia, Moscow, Russian Federation*, <sup>2</sup>*Russian Clinical Child Hospital, Moscow, Russia, Moscow, Rus-*

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We describe clinical data of 9 patients with mutations in the gene, coding the mitochondrial polymerase gamma (POLG). Mutations in the POLG gene are one of the most common causes of mitochondrial disease in children and adults.

We investigate 3 adult patients with clinical picture of mitochondrial disease. One patient, a 62-old woman with severe ataxia, external ophtalmoplegia, paresthesia in legs, autoimmune thyroiditis and myopathy. She has W748S mutation in POLG gene. Two patients had A467T and P587L mutations consequently and showed typical symptoms of CPEO.

We investigate six children with hepatopathy and myopathy.

Four patients from this group had Alpers syndrome: intractable seizures with a focal component, severe neurological deterioration, hepatic failure. Additional symptoms were vomiting, ataxia, hypotonia, high levels of lactate, liver transaminase and high concentration of tyrosine in urine. Mutant POLG alleles were detected in all Alpers syndrome cases (G268A/A467T; W748S/G848S; W748S/L311P; W748S/T855S). Symptoms of the rest two patients weren't suitable for classical Alpers syndrome. They both didn't have epilepsy, but have very severe hypoglycemia with severe hepatic failure and muscle hypotony. We revealed only one mutant POLG allele in each case, A467T and W748S consequently. mRNA analysis didn't detect exon deletions in POLG gene. The presence of the second mutant allele in the promotor region or locus heterogeneity involving mutations in other "mitochondrial" genes can not be excluded.

Clinical phenotypes of POLG-related disorders are characterized by extremely variability of symptoms and this group may be more common than previously thought.

**P13.03****Evaluation of disease relevant mutations in Anderson Fabry Disease - how to proof the medical consequences?**

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Anderson Fabry Disease (AFD) is an X-linked hereditary disorder of sphingolipid metabolism caused by a genetic defect of the lysosomal hydrolase  $\alpha$ -galactosidase A (AGLA). So far, more than 300 AGLA missense mutations have been described. However, numerous amino acid exchanges appear to have only mild effects on enzyme activity reduction.

Bioinformatic tools cannot reliably predict the effects of these changes and inevitably need to be supported by enzymatic measurements. Therefore, a rapid screening procedure for all AGLA mutations will be invaluable for this purpose. To solve the limitations of all routine methods we developed an *in vitro* model based on the overexpression of AGLA mutants using a HEK293 cell system. Using fluorescence enzyme activity assays we are able to determine the activity of mutant enzymes compared to the wildtype. This procedure has shown that the following mutations, which are described in the literature or have been found in our screening programs for Fabry have to be interpreted as SNPs and not clinically relevant AGLA mutations: D83N, S102L, S126G, R220Q, R252T.

Tools for the systematic *in-vitro* evaluation of newly detected mutations are also of high value for the evaluation of the consequences of new therapeutic strategies, e.g. using pharmacological chaperones (PCs) such as 1-Deoxygalactonojirimycin (DGJ). One of the challenges is to find the right PC for any particular mutation because a substance might perform well on one mutation while failing on another. The present study includes a solution for screening newly synthesized drugs to discover substances capable of acting like PCs.

**P13.04****The molecular landscape of genetic B12 metabolism disorders in patients from Mediterranean and Latin- American origin**

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Centro de Biología Molecular. Universidad Autónoma de Madrid, Madrid, Spain. Vitamin B<sub>12</sub> is the cofactor of Methionine synthase (MTR) and Methylmalonyl-CoA Mutase (MCM) that catalyze the remethylation of homo-

cysteine and the isomerization of L-methylmalonyl-CoA, respectively. To date defects in ten different genes coding for proteins involved in B<sub>12</sub> transport, synthesis of active cofactors-MetCbl and AdoCbl- and for the two apoenzymes MCM and MTR have been described. We report the molecular analysis of 65 cases with isolated methylmalonic aciduria (MMA) and 32 with methylmalonic aciduria combined with homocystinuria (MMACH) mainly from Mediterranean and Latin-American origin referred to our laboratory. Using cellular, enzymatic and genetic approaches the MMA patients were classified belonging to the *cblA* (n=13), *cblB* (n=6), *cblD*-variant2 (n=3) and *mut* (n=43) complementation groups, and the MMACH patients belonging to the *cblC* group (n=32). The mutational spectrum in the *mut* affected patients includes a high frequency of missense changes (56%). Two deep intronic changes causing intronic sequence exonization were found and *in vitro* antisense therapy was investigated. In *cblB* affected patients intronic and exonic nucleotide changes affecting splicing were frequent, and two misfolding missense mutations have been functionally analyzed. Finally, in *cblA*, *cblC* and *cblD*-variant2 complementation groups a high proportion of premature stop codon changes (PTC), including nonsense and frame-shift mutations, has been identified. In *cblC* type patients the highest frequency described to date of the common change R91fs (89%) has been found. The present work has a diagnostic and therapeutic value in this complex metabolism addressing our investigation towards genetic specific therapies.

**P13.05****Study of apoptosis in genetic B<sub>12</sub> metabolism disorders (cblB and cblC types)**

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B<sub>12</sub> metabolism disorders are a heterogeneous group of rare genetic metabolic diseases caused by defects on cobalamin adsorption, transport or function. The aim of this study was to analyze apoptosis in several patients' fibroblasts with defects on two of the genes involved (MMAB and MMACHC) by flow cytometry, as well as to investigate the differential gene expression in pathways that regulate this process by PCR array. Patients' cell lines showed a higher rate of apoptosis compared to controls. Using the human apoptosis pathway array, we examined the gene expression profiles exhibited by six *cblC* and two *cblB* patients' fibroblasts relative to four control individuals. Of the 84 apoptosis pathway-focused genes in the array, a total of 31 genes were differentially expressed. Up-regulation was observed in 23 genes, while 8 genes appeared to be down-regulated in patients' fibroblasts. The most highly up-regulated genes belong to BCL2 and TNF receptor and ligand functional gene families. Multiple anti-apoptotic genes, including BAG1, BCL2, BCL2A1, BCL2L1, NAIP, BRAF, MCL1, TNF, CD27 and IGF1R were up-regulated; meanwhile BFLAR was down-regulated. PCR array results are currently being confirmed by Western Blot. In addition, we have detected the up-regulation of the intracellular modulators of apoptosis, JNK and p38 MAP kinases, in patients' cells by immunoblotting. In conclusion, the increased rate of apoptosis in *cblB* and *cblC* patients' fibroblasts might up-regulate the anti-apoptotic molecules representing a compensatory response to a potential disease pathogenic mechanism.

**P13.06****Novel mutation in the GCH1 gene causing GTP-6 cyclohydrolase deficiency in two saudi sibs and early detection by serum neopterin**

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GTP-6 cyclohydrolase (GTPC) deficiency is a rare autosomal recessive disorder of the terahydrobiopterin (BH4) pathway. Only 5 patients reported in the literature. GTPC deficiency characteristic by hyperphenylalaninemia and low concentration of neopterins in urine and blood and neurotransmitter defect. We report tow sibling diagnosed with GTPC deficiency, one late and other early diagnosis. Patient 1 presented at the age of nine years with severe global development delay and frequent twitching of body. The plasma amino acid showed a phenylalanine of 357 umol/l. Which was not consistent with classical

PKU. BH4 challenge test showed normalization of phenylalanine indicating BH4 dependent PKU. Serum neopterin level was extremely low at < 2.0 (2.0-10 nmol/L) suggesting that the cause is most likely GTPC deficiency. Patient was started on BH4, L-dopa/carbipoda, 5-hydroxy-typtophan, and folic acid without appreciable improvement.. Patient 2 is the brother who at age of 3 months and in view of positive family history of GTPC deficiency, serum neopterin level was found to be low at <2 (2.0-10 nmol/L) confirming the suspicion about the disease. He immediately initiated on therapy. Further confirmation was accomplished by full sequencing of the *GCH1* gene, and a novel homozygous D237V mutation in exon 6 of the gene was found. Now 5 years old, he has normal gross and fine motor skills with borderline cognitive delay. To our knowledge, D237V is a novel mutation that has not been previously reported in the literature, and the two sibs are the first confirmed patients with GTPC deficiency in Saudi Arabia.

#### P13.07

#### The frequency of 21 hydroxylase gene defects, phenotypic effects and other molecular mechanisms in congenital adrenal hyperplasia patients in Turkish population.

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Introduction: Congenital adrenal hyperplasia (CAH) is a genetic, endocrine and metabolic disorder, caused by mainly 21-hydroxylase enzyme deficiency.

CAH, due to 21 hydroxylase deficiency can be divided into two groups as classic and non-classic type. In classic type most patients can not synthesize sufficient aldosterone to maintain sodium balance and may develop potentially fatal "salt wasting" crises if not treated.

Methods: To determine the frequency of 21 hydroxylase gene mutations in Turkish population, CYP21A2 gene regions starting from promoter to the end of exon 4 were analyzed in 90 patients and 80 controls. DNA isolation from peripheral blood was performed and CYP21A2 gene was amplified with specific primers for the functional gene by PCR. Mutations were detected by using direct sequencing after the purification of amplified DNA. Results were evaluated statistically.

Results: We found one, one and three novel mutations in promoter region, exon 1 and intron 2; also three and one novel polymorphisms in exon 1 and intron 2 respectively. Additionally we found 17 base substitutions. In patients, 2 mutations found in intron 2 are significant compared to controls. There are 26 significant correlations between base substitutions. We found 5 significant base substitutions between clinical phenotypes. When patients are compared with controls for the regions, intron 2 is significant for base substitutions, intron 2 and intron 3 are significant for phenotypes.

Conclusion: In our study, 26 base substitutions were found including novel ones. To our knowledge, this is the first data obtained from direct sequencing of Turkish CAH patients.

#### P13.08

#### A novel mutation in *LMBRD1* causes the cbfL defect of vitamin B<sub>12</sub> metabolism in a Turkish patient

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In the cbfL defect of vitamin B<sub>12</sub> (cobalamin) metabolism, cobalamin is trapped in lysosomes. Consequently, cobalamin coenzyme synthesis is blocked and cofactors for methionine synthase and methylmalonyl-CoA mutase are deficient. We recently identified *LMBRD1* as the cbfL associated gene located on chromosome 6q13 and showed that 18 out of 24 alleles in unrelated patients carried the deletion c.delG1056 (p.L352fsX18). *LMBRD1* encodes a lysosomal membrane protein,

which facilitates lysosomal export of cobalamin.

Our patient is the second child of consanguineous Turkish parents. He presented on the second day of life with cerebral seizures and intraventricular hemorrhage. Plasma homocysteine and urinary methylmalonic acid levels were elevated, serum cobalamin levels were decreased. Cultured skin fibroblasts showed deficient synthesis of both cobalamin coenzymes. The cbfL defect was confirmed by somatic complementation analysis. Sequencing of *LMBRD1* revealed the novel deletion c.delG1405(p.D469fsX38) in exon 14 on both alleles. Real-time PCR revealed mRNA levels comparable to those in controls, which is in contrast to the reduced levels found in the patients with the common deletion. Transfection of patient fibroblasts with the *LMBRD1* wild type cDNA rescued coenzyme synthesis confirming this new deletion as an additional cause of the cbfL-defect.

This case adds to our knowledge of the spectrum of clinical presentation and mutations of this rare disorder of lysosomal transport.

#### P13.09

#### Detection of new mutations in thyrotropin receptor gene in children with congenital hypothyroidism

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The TSH receptor gene (*TSHR*) encodes a transmembrane receptor present on the surface of follicular cells which mediates the effects of TSH secreted by the anterior pituitary and is critical for the development and function of the thyroid gland. It belongs to a subfamily of heptahelical G protein coupled receptors that have a common structure consisting of seven transmembrane segments, three extracellular and three intracellular loops, an extracellular amino terminal domain, and an intracytoplasmic carboxyl terminal tail. We examined the frequency of *TSHR* mutations among patients with permanent primary congenital hypothyroidism and in the general population of Republic Bashkortostan (Russia).

We enrolled 63 patients with primary congenital hypothyroidism who identified through newborn screening.

We investigated all 10 exons of *TSHR* gene and detected 3 mutations including one well characterized substitution R450H and two new heterozygous mutations.

Those 3 patients had markedly elevated TSH levels concomitant with low peripheral thyroid hormone levels and a reduced thyroid volume as determined by ultrasonography. This constellation of clinical findings warranted the diagnosis of congenital hypothyroidism.

In one case was detected a heterozygous mutation R450H in the *TSHR* gene. Nagashima et al. are reported that the R450H mutants demonstrates a slightly impaired receptor function.

Also was found heterozygous mutation C1318A which resulted in a L440I substitution of the *TSHR* protein.

A novel heterozygous cytosine to thymine transition at nucleotide position 1591 in 10 exon (R531W) was found in the third case.

So, additional investigations are needed for conformation of pathogenicity for this novel sequence variations.

#### P13.10

#### The first founder *DGUOK* mutation associated with hepatocerebral mitochondrial DNA depletion syndrome

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Deoxyguanosine kinase (dGK) deficiency is a frequent cause of mitochondrial DNA depletion associated with a hepatocerebral phenotype. In this study, we describe a new splice site mutation in the *DGUOK* gene and the clinical, radiologic, and genetic features of these *DGUOK* patients. This new *DGUOK* homozygous mutation (c.444-62C>A) was identified in three patients from two North-African consanguineous families with combined respiratory chain deficiencies and mitochondrial DNA depletion in the liver. Brain MRIs are normal in *DGUOK* patients in the literature. Interestingly, we found subtentorial abnormal myelination and moderate hyperintensity in the bilateral pallidi in our patients. This new mutation creates a cryptic splice site in intron 3 (in

position -62) and is predicted to result in a larger protein with an in-frame insertion of twenty amino acids. *In silico* analysis of the putative impact of the insertion shows serious clashes in protein conformation: this insertion disrupts the  $\alpha$ 5 helix of the dGK kinase domain, rendering the protein unable to bind purine deoxyribonucleosides. In addition, a common haplotype that segregated with the disease in both families was detected by haplotype reconstruction with ten markers (microsatellites and SNPs), which span 4.6 kb of DNA covering the *DGUOK* locus. In conclusion, we report a new *DGUOK* splice site mutation that provide insight into a critical protein domain (dGK kinase domain) and the first founder mutation in a North-African population

#### P13.11

#### High incidence of Fabry Disease - causing mutations in Galicia, North-west Spain

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Fabry disease (FD) is an X-linked lysosomal storage disorder that results from mutations in the  $\alpha$ -galactosidase A ( $\alpha$ -Gal A) gene which lead to a deficient activity of the enzyme and a progressive lysosomal deposition of globotriaosylceramide and other glycosphingolipids in different cells of the body.

In its classic form, with a typical onset in childhood or adolescence, the major disease manifestations include angiokeratoma, acroparesthesias and vascular disease of the heart, kidneys and brain.

The bibliography shows an estimated incidence of 1/117000 newborns, representing the second most prevalent lysosomal lipid storage disease. However, recent studies suggest that FD is underdiagnosed demonstrating that neonatal incidence may fluctuate between 1/3100 and 1/4600.

#### Material and methods.

We designed primers for the direct bidirectional sequencing of the coding region of the  $\alpha$ -Gal A gene and carried out an study of the mutations of the gene in 112 newborns with low  $\alpha$ -galactosidase A activity (less than 2  $\mu$ mol/L/h) measured by fluorimetry.

#### Results.

In the sample of 112 newborns, we found 10 unrelated cases with pathological missense mutations (R118C: 5 hemizygous males; A143T: 4 hemizygous male and 1 heterozygous female). In addition, 4 previously undescribed intronic variants surrounding intron-exon boundaries were identified in six cases.

Since the disease follow an X-linked dominant mode of inheritance (heterozygous females typically have milder symptoms at a later age of onset than males) these results demonstrate an unusual neonatal incidence of 1/1400 in the North-West Spanish population of Galicia, the highest Fabry disease rate known to us in the literature.

#### P13.12

#### Two different related Fabry mutations in the same family: "Who isn't saying the truth?"

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Introduction Fabry disease (FD) is an X-linked lysosomal  $\alpha$ -galactosidase A deficiency that causes multisystemic problems, including renal, neurological, ocular, skin, and cardiac manifestations.

Methods. A 43 year old female, with arterial hypertension, was remitted to our clinic because of a suspected diagnosis of hypertrophic cardiomyopathy. Clinical, electrocardiographic, echocardiographic, plasma  $\alpha$ 1-galactosidase A activity and genetic study was made of all available relatives.

Results. The index case echocardiogram shown left ventricular hypertrophy mainly of posterior-lateral wall (16mm). Her adolescent son had been diagnosed of erythromelalgia, with cutaneous lesions consistent with angiokeratomas and suffering for intense pains in his feet and hands (the mother had suffered the same pains in her youth). The son also presented mild left ventricular hypertrophy (13mm, postero-basal

wall). A probably FD with the classical form of the disease was suspected in both, due to a low plasma  $\alpha$ 1-galactosidase enzymatic activity. Moreover, her aunt and her cousin, diagnosed of a mild FD form, presented the A143T mutation in alpha-galactosidase A gene (GLA). We suspected that the mutation A143T in the GLA was responsible for the disease in the new cases. However, the genetic analysis showed that they were not carriers of the A143T mutation but they presented the mutation IVS5+2T>C previously associated with the classic form of FD. This intronic mutation was not present in the patient with the A143T mutation.

Conclusion. In this family we described two mutations previously associated with FD, but with available data we think that the pathogenicity of the A143T mutation must be reevaluated.

#### P13.13

#### Five novel mutation in Fabry disease in a series of Russian patients.

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Fabry disease is an X-linked recessive abnormality related to lysosomal storage disorders. It is caused by deficiency of  $\alpha$ -D-Galactosidase A ( $\alpha$ -GAL). Main symptoms include renal failure, cardiovascular disease, angiokeratoma, acroparaesthesia. In this issue we include 10 male patients with classical clinical picture of Fabry disease.  $\alpha$ -GAL activity in leukocyte ranges from 0,01-7 nmol/mg/h. Using the adopted method we measured the  $\alpha$ -GAL activity in dried blood spots (DBS) in 5 patients. Enzyme activity in DBS range from 0-0,04 nmol/spot\*45h. The enzyme deficiency was detected in all samples. We made sequences of GLA gene; mutations were revealed in all cases and were private for each family. Five mutations have been already described (R112C, Cys142R, R227X, Thr282P, c.238delAA). And the rest five are novel (R301L, c.782delG, Cys63R, R220X, c.541del18). Biochemical assay combined with DNA-testing gives the most effective diagnostic approach. DNA analysis is preferable in Fabry carriers as the  $\alpha$ -GAL activity could be at the cut-off range in this group.

#### P13.14

#### Acute Abdomen Reasoned Surgery Frequency and MEFV Mutations in the Patients with FMF

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Familial Mediterranean Fever (FMF), is an autosomal recessive disease characterized by recurrent fever, peritonitis, arthritis, pleuritis and neuropathic amyloidosis. The disease is common in North African and Iraqi Jews, Turks, Armenians, Middle East Arabs and in ethnic groups living in this region. More than 95% of FMF patients have peritoneal involvement mimicking acute abdomen, and sometimes this involvement causes unnecessary surgical intervention. In the current study we objected to determine the frequency of acute surgical abdomen intervention and MEFV gene mutations in FMF patients.

In the interview of 159 patients referred to our department by prediagnosis of FMF, totally 26 (16.4%) were detected to be operated. Of these 17 (10.7%) were operated by the reason of appendicitis and 9 (5.7%) were operated by other acute abdomen reasons. When the mutations of these patients evaluated, M694V(40.5%) and E148Q (21.4%) mutations were the most frequent ones.

The mutation frequency in FMF patients with acute surgical abdomen intervention (80.8%) was significantly high than the ones without (56.4%). The increase of mutation scanning in FMF patients will significantly decrease unnecessary surgical intervention in this patient group.

#### P13.15

#### The bone mineral density in patients with Familial Mediterranean Fever

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FMF is hereditary illness. Although it was reported in one study that bone mineral density was decreased in child patients with FMF, but the effect of FMF on BMD is not known in adult patients. The aim of this study is evaluation of the effect of FMF on BMD in adult patients.

Based on the Livneh criteria 23 FMF patients and control group with 15 people admitted to the study. Exclusion criteria were taking steroid for a long time, chronic renal failure, woman in post menopause. The body mass index, BMD (between L1-L4), and Z scores were measured in both groups. In addition to this taking drug period and illness duration were taken into consideration in FMF patients. BMD and the other parameters were compared between groups. After that BMD related parameters were evaluated.

The mean age of FMF patients (10-female, 13-male) and control group (10-female, 5-male) was  $30.5 \pm 8.2$ (18-48) years and  $35.0 \pm 10.2$ (22-54) years respectively. All of the women in both groups were premenopausal. There was no difference between two groups for the mean age, gender and BMI. But FMF patients have had statistically significant reduction in BMD (between L1-L4) and Z-scores ( $p=0.015$  and  $p=0.010$  respectively). No statistically significant correlation was found between BMD and age, BMI, illness duration in the FMF population. FMF that have episodic inflammation bring about to decrease in BMD. In our study we didn't find any factor effective on BMD. Factors that could be affecting the BMD should be research in the big population.

#### P13.16

##### Variants in the FTO gene associate to hyperandrogenemia and metabolic parameters in polycystic ovary syndrome

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**Background:** Variants in the fat-mass and obesity associated gene (FTO) are associated with obesity and type 2 diabetes. Women with polycystic ovary syndrome (PCOS) frequently present with obesity and impaired glucose tolerance. The aim of this study was to investigate the impact of FTO variants on metabolic and endocrine parameters in PCOS women.

**Methods:** We genotyped single nucleotide polymorphism rs9939609 (T/A) in 179 PCOS women and 126 female controls. We performed metabolic and hormonal measurements, oral glucose tolerance test, hirsutism score, and lipometry.

**Results:** The A allele was associated with significantly increased free testosterone ( $p=0.026$ ), FAI ( $p=0.042$ ), weight ( $p=0.006$ ), BMI ( $p=0.008$ ), waist ( $p=0.035$ ) and hip circumference ( $p=0.041$ ), and triglycerides ( $p=0.032$ ). In logistic regression, the A allele was associated with free testosterone ( $p=0.032$ ; OR 1.7; 95% CI 1.05-2.9; B=0.76). Total fat mass ( $p=0.007$ ), visceral adipose tissue mass ( $p=0.013$ ), subcutaneous fat mass ( $p=0.007$ ), and lean body mass ( $p=0.007$ ) were significantly increased in PCOS women carrying the A allele. The A/T+A/A genotype showed increased prevalence in overweight/obese PCOS patients (OR=2.5;  $p=0.006$ ) and in PCOS women with impaired glucose tolerance (OR=3.6;  $p=0.017$ ). No significant association was seen between FTO variants and the PCOS per se.

**Conclusion:** In summary, we demonstrated that FTO variants influence hyperandrogenemia and anthropometric parameters in women with PCOS, indicating an important role of FTO variants not only in obesity and diabetes but also in hyperandrogenism in women with PCOS.

#### P13.17

##### Biochemical and molecular diagnosis of Galactosemia in Iranian patients

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Classical galactosemia is an inborn metabolic disorder caused by autosomal recessive mutations in galactose 1 phosphate uridyl transfer-

ase (GALT) gene. The incidence of the disease varies from 1:40000-1:60000 among Caucasian population. To date, over 170 different mutations have been reported including point mutations, microdeletions and insertions. The most frequent reported mutation is Q188R which accounts for approximately 60% of mutant alleles in Caucasian population.

In the present investigation, results of a 5-year study on galactosemia in Iran including biochemical diagnosis and molecular analysis of GALT gene are presented.

**Methods:** Twenty five galactosemia patients were subjected to diagnosis

of galactosemia by the determination of GALT activity in RBCs using Beutler test. DNA samples were investigated for the 5 most reported mutations including Q188R, K285N, X380R, L195P and Q169K using PCR-RFLP method. PCR-SSCP

method was used for the whole GALT gene including 11 exons and flanking intronic sequences to investigate the mutations which were not detected by PCR-RFLP method. In a retrospective study, galactosemic patients were traced and their long term outcomes were evaluated.

**Results:** Q188R mutation was the most observed mutation with the allelic frequency of 57.1%. The allelic frequencies for S135L, Y209S, A320T, and K285N were found to be 7.1%, 7.1%, 7.1%, and 3.57%, respectively.

**Conclusion:** Our results show that galactosemia is a heterogeneous disorder at the molecular level in Iranian population. Study on long term outcome of the disease emphasizes the need for a new look and new challenges for galactosemia in Iran.

#### P13.18

##### Gamma-Hydroxybutyric aciduria - identification of first Bulgarian case by a metabolomic approach

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Succinic semialdehyde dehydrogenase (SSADH) deficiency (MIM 271980) is a rare autosomal recessive disorder due to defect in gamma-aminobutyric acid catabolism, resulting in the accumulation of gamma-hydroxybutyric acid (GHB) and causing neurological disorders of varying severity. SSADH is characterized by psychomotor retardation, childhood-onset hypotonia, and ataxia. Seizures occur in more than 50% of affected individuals. The diagnosis of SSADH deficiency is suspected in individuals with gamma-hydroxybutyric aciduria present on urine organic acid analysis and can be confirmed by assay of SSADH enzyme activity in leukocytes. ALDH5A1 is the only gene currently known to be associated with SSADH deficiency.

We report an 11 years old boy, fifth child of a consanguineous roma family. The patient presented with severe psychomotor retardation after birth. Later on he developed seizures. His older sister, the fourth child of the family has similar clinical signs of profound mental retardation and seizures. The boy died with severe dehydration and epileptic status during an ongoing infection.

Preinvestigation findings were abnormal urine ketones; metabolic profile by GC/MS organic acid analyses in urine showed marked excretion of GHB and 3,4-dihydroxybutyric acid. Ketonuria and dicarboxylic aciduria were detected. SSADH enzyme activity have not been investigated because leukocytes are not available.

Substantial excretion of GHB against the background of the family history and the clinical phenotype suggest succinic semialdehyde dehydrogenase deficiency. The significant amounts of dicarboxylic acids in the urine may indicate a secondary inhibition of mitochondrial fatty acid beta-oxidation or propionyl-coenzyme A metabolism by succinic semialdehyde or its metabolites.

#### P13.19

##### Gaucher's disease type I - cytokines profile

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**Aim:** The paper proposed to investigate the dynamics of the serum levels of cytokines in a group of patients with Gaucher disease in correlation with the severity score index and skeletal involvement. **Patients and methods:** The group included 15 patients with Gaucher disease aged between 10-53 years; the diagnosis was based on clini-

cal, enzymatic and molecular tests and 15 controls. The severity score index of the disease, the clinically and radiologically documented bone involvement and the degree of the osteopenia (DEXA method) were determined in all patients. The cytokine profile (IL-1beta, IL-6, IL-8, IL-10, TGF-beta) was determined using ELISA (Quantikine Human RD System). The assessments were made at baseline and one year later. **Results:** Baseline levels were different only for IL-6 (10.5 +/- 3.11 ng/ml vs 1.48 +/- 1.13 ng/ml in controls) and IL-10 (15.6 +/- 5.59 ng/ml vs 5.07 +/- 1.08 ng/ml in controls). The analysis of the data in dynamics did not evidence changes in the cytokines profile, with the exception of TGF-beta (18.97 +/- 7.03 ng/ml vs 10.97 +/- 2.35 ng/ml). A significant correlation was found between the IL-6 serum level and the bone score ( $r = 0.823$ ,  $p = 0.007$ ), but no correlation between the cytokines level and the spinal bone mineral density. **Conclusions:** The serum profile of cytokines might be useful to investigate the pathological mechanisms of bone changes in Gaucher disease.

#### P13.20

##### Dyslipoproteinaemia in Gaucher disease: new study over 44

##### Romanian patients

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Gaucher disease has, among metabolic complications, a specific dyslipoproteinaemia. This study evaluates the lipid profile and the impact of apolipoprotein E gene polymorphism on this metabolic status. Forty-three type I and one type III Gaucher patients, 33 receiving ERT and 11 nontreated, were analyzed during a period of 3.6 +/- 1.21 years and respectively 3.8 +/- 2.2 years. Total cholesterol, fractions HDL and LDL-cholesterol, tryglicerides, apolipoproteins A, B and E were assessed every 6 months, using standard methods.

Results are summarized in table I.

No	Parameter	Reference value	Treated patients (n=33)			Untreated patients (n=11)		
			Initially	Finally	Point of significance (p<0.05)	Initially	Finally	Point of significance (p<0.05)
1	Total cholesterol	percentile 5, related to age and sex	-4%	+30%	at 1.5 years of ERT	-9%	+3%	none
2	HDL-cholesterol	percentile 5, related to age and sex	-16%	+52%	at 1 year of ERT	-5%	-7%	none
3	LDL-cholesterol	percentile 5, related to age and sex	-5%	+37%	at 1.5 years of ERT	-7	+17%	none
4	Tryglicerides	percentile 95, related to age and sex	+4%	-23%	at 1 year of ERT	-18%	-14%	none
5	Apolipoprotein A	minimum: 106 mg/dl	-74%	+36%	at 1.5 years of ERT	-13%	+24%	none
6	Apolipoprotein B	minimum: 56 mg/dl	+6%	+89%	at 1 year of ERT	+76%	+62%	none
7	Apolipoprotein E	maximum: 5 mg/dl	5.43 x <sup>1</sup>	5.17 x <sup>2</sup>	none	10.04 x <sup>3</sup>	6.14 x <sup>4</sup>	none

Table I. Evolution of lipid profile in Gaucher patients (at: <sup>1</sup>3 +/- 1.1, <sup>2</sup>3.5 +/- 1.2 years of ERT ; <sup>3</sup>1.7 +/- 2.3, <sup>4</sup>3.8 +/- 2.2 years of monitoring)

The apo E polymorphism analysis, performed by PCR-RFLP, in 39 patients, revealed the following genotypes:  $\epsilon 3/\epsilon 3$  (77%),  $\epsilon 3/\epsilon 4$  (12.8%) and  $\epsilon 2/\epsilon 3$  (10.2%). The presence of  $\epsilon 2$  allele significantly changed the lipid profile into a type III hyperlipoproteinemic pattern (after receiving ERT). Presence of  $\epsilon 4$  allele did not impact on total cholesterol and LDL-cholesterol level. Apo E levels in  $\epsilon 2/\epsilon 3$  carriers were twice higher than those in  $\epsilon 3/\epsilon 4$  carriers, significantly correlated with tryglicerides' level.

Conclusion: Gaucher disease patients presented a potentially pro-atherogenic dyslipoproteinaemia (hypcholesterolemia, hypo-HDL, hypo-LDL-cholesterolemia and hypertrygliceridemia), significantly improved by enzyme replacement therapy with imiglucerase. Plasma levels of apolipoprotein E still remained elevated after receiving 3.5 years of ERT. Genotype  $\epsilon 2/\epsilon 3$  validates into phenotype rather than  $\epsilon 3/\epsilon 4$  genotype, in treated Gaucher patients.

#### P13.21

##### A novel SLC2A1 mutation causing paroxysmal exercise-induced dyskinesia in twin Spanish patients

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**Introduction:** Paroxysmal exercise-induced dyskinesia (PED) is a rare disorder featuring brief, dystonic or choreoathetotic attacks, triggered by prolonged exercise, often occurring in association with different types of epilepsy. Familial cases suggest autosomal dominant inheritance. Two recent studies have identified mutations in the *SLC2A1* gene, encoding the GLUT1 glucose transporter, in patients with PED. **Objective:** To study a family with monozygotic twins presenting with PED, learning disability and childhood absence epilepsy (CAE) at age 5 years.

**Patients and Methods:** In both patients, aged 9, PED episodes occurred daily and improved rapidly upon intake of isotonic beverages. The EEG showed generalized paroxysmal 3 Hz spike-wave discharges; seizures improved with ethosuximide. The CSF/serum glucose ratio was low at 0.4 in both children. The 10 exons of the *SLC2A1* gene and their corresponding exon/intron junctions including splice sites and branch points were PCR amplified and sequenced.

**Results:** The new mutation c.G493A was identified in exon 4 of *SLC2A1*, leading to a p.Val165Ile substitution in GLUT1 protein, in the two siblings but not in their parents. The replaced valine residue is highly conserved in evolution. The mutation was not present in one hundred unrelated healthy Spanish individuals. An identical mutation described by others in the paralogous GLUT2 is known to markedly reduce glucose uptake.

**Conclusion:** We report a novel mutation in the *SLC2A1* gene in twin patients with PED and CAE. This finding confirms and extends previous observations indicating that GLUT1 deficiency must be considered in the diagnostic work-up of paroxysmal movement disorder-epilepsy syndromes.

#### P13.22

##### Glycerol kinase deficiency

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Glycerol kinase deficiency (GKD) is an inborn error of metabolism that was initially described in 1977. Glycerol kinase (GK) catalyzes the phosphorylation of glycerol to glycerol-3-phosphate in ATP-dependent reaction. Affected individuals present with hyperglycerolemia and glyceroluria. GKD presents with diverse phenotypes and is categorized into three different clinical presentations: - Infantile or complex glycerol kinase deficiency (cGKD)- the most common form, a contiguous gene deletion syndrome caused by a loss of glycerol kinase (MIM# 300474), along with its neighboring genes, Duchenne muscular dystrophy (DMD; MIM# 300377) and/or Nuclear Receptor Subfamily 0, Group B, Member 1 (NR0B1; MIM# 300473) - Juvenile or symptomatic results in point mutation in GK that causes episodic metabolic and central nervous system decompensation - Adult form resulting from point mutation detected incidentally with pseudohypertriglyceridemia. The authors present 4-month-old boy with cGKD coupled with liver dysfunction. Measured activity of glycerol kinase in peripheral blood leucocytes (by incorporation of <sup>14</sup>C glycerol carbon into trichloroacetic acid method) was residual - 1,3% of control value (0,84 pmol/min/mg of protein, reference: 60-178 pmol/min/mg of protein). Mutation study revealed deletion involving the whole GK loci as well as loci for DMD which confirmed the diagnosis of GKD.

#### P13.23

##### Mouse models for glycogen storage disease type III and IV

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Glycogen storage disease type III (GSD-III) and IV (GSD-IV) are autosomal recessive diseases resulting from deficient glycogen debranching enzyme (AGL) and glycogen-branching enzyme (GBE) activity

respectively. GSD-III is characterized by excessive accumulation of abnormal glycogen with short outer chains, in the liver and/or skeletal and cardiac muscles. GSD-IV is characterized by the accumulation of amylopectin-like polysaccharides. The typical presentation is liver disease of childhood and progressing to lethal cirrhosis. GSD-III and GSD-IV are clinically heterogeneous disorders.

Using a gene-driven ENU-mutagenesis approach, the mice carrying the missense mutation T531M in AGL gene, or the stop codon mutation E609X in GBE1 gene have been generated respectively. The homozygous GSD-III T531M mice appeared normal at birth. Glycogen highly accumulated in the liver of the 20-week-old homozygous GSD-III T531M mice was observed. The mice carrying the homozygous E609X stop codon mutation in the GBE1 gene showed perinatal death. The heartbeats in some homozygous GSD-IV E609X mutation fetus stopped at around 10.5 days of gestation, and the histopathology studies from the fetus revealed no glycogen accumulated in the wall of heart chamber and liver primodium, however glycogen is significant accumulated in these regions at the same stage of wild type fetus. In the new born GSD-IV E609X mice the diastase-resistant PAS positive material was observed in the liver and cardiomyocytes.

These mice represent important animal models for the study of abnormal glycogen metabolism and its related toxicity and to investigate pathophysiology and treatment strategies for human GSD-III and GSD-IV.

#### P13.24

#### Hereditary folate malabsorption: case report and response to treatment with folic acid

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Hereditary folate malabsorption (HFM) is a rare autosomal recessive disorder in which folic acid cannot be absorbed from the gastrointestinal tract or transported from the blood stream into the brain. As a result, patients present with failure to thrive, megaloblastic anemia, and, without treatment, progressive neurologic deterioration. A defective folate transport protein (Proton-coupled folate transporter, PCFT) was recently identified as the cause of the disorder. Less than 20 cases of HFM have been reported in the literature, most of them female. We report another case in whom treatment with folic acid resulted in improved growth and stabilization of neurologic status.

A 12 month old girl, product of consanguineous parents, was referred for evaluation of seizures and occipital calcifications. Despite these findings, her development was normal. The past medical history was significant for failure to thrive and for megaloblastic anemia at 3 months of age that responded only partially to treatment with folic acid. CSF 5-methyltetrahydrofolate, a folate metabolite, determination was <2 nmol/L (normal 40 - 187 nmol/L), consistent with the diagnosis of HFM.

Initiation of treatment with IM folic acid resulted in dramatic improvement in her growth parameters and stabilization of her neurologic status, despite the fact that CSF levels never reached the reference range of the testing laboratory. To date, she has had no further seizures and, aside from mild language delays, has exhibited normal growth and development. These results emphasize the importance of accurate diagnosis and early treatment in patients with this disorder.

#### P13.25

#### Mutant HFE genotype leads to significant iron overload in patients with liver diseases from western Romania

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Hereditary haemochromatosis (HH) is an autosomal recessive disorder of iron metabolism affecting 0.4% - 0.7% of European populations. Approximately 80% of affected individuals are homozygous for the C282Y mutation, but the H63D and S65C mutations are also of interest. The clinical penetrance and frequency of complications of HH is a subject of controversy. The present study aimed at assessing the HFE mutations (C282Y, H63D and S65C) in western Romanian patients with liver disease of diverse aetiologies suspected of iron overload. A total of 21 patients, all Romanian residents hospitalized with clinical

suspicion of iron overload and liver disease, were assayed for C282Y, H63D and S65C mutations, serum ferritin, transaminases, and viral hepatitis markers. Overall, 9 out of the 21 patients (42.86%) were found harbouring mutations in the HFE gene: four C282Y homozygotes (19.0%), one compound heterozygote C282Y/H63D (4.8%), one single heterozygote C282Y (4.8%), two single heterozygotes H63D (9.5%), one single heterozygote S65C (4.8%), and 12 wild-type cases (57.1%). Among the subgroup of 10 patients with the most prominent signs of iron overload (hyperferritinemia and/or hepatocyte iron score  $\geq 1$ ), without hepatocellular carcinoma, the HFE genotypes were conclusive in 5 cases (50%). They had significantly increased ferritin levels compared to wild-type cases ( $P = 0.029$ ). The inclusion of iron studies during routine clinical visits coupled with the availability of HFE genotyping for family and population studies will facilitate the early detection of HH in Romania.

#### P13.26

#### Molecular genetic analysis of multiple osteochondromas in Bulgarian patients

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The authors present two clinical cases with Multiple osteochondromas /MO;exostoses/-a metabolic disease with autosomal dominant inheritance caused by an O-xylosylglycan defect of protein O-glycosylation and characterized by bony outgrowths mainly located in the juxta-epiphyseal region of the long bones . The DNA analysis was performed by PCR amplification and direct sequencing of all coding exons /exon 1-11/ of the EXT1 gene and / exon 2-14/ of the EXT2 gene and MPLA analysis.The first child harbours a c.1468del C(p. Leu490TrpfsX9)in exon 6 of the EXT1 gene which causes a frameshift and premature stop codon , resulting in the formation of an unstable mRNA or a truncated non functional protein.The second child harbours a c.1079+1G>T mutation in intron 6 of the EXT2 gene which affects most likely proper splicing of exon 6 , resulting in the formation of an unstable mRNA or a truncated non-functional EXT1 protein.The DNA analysis was performed for a first time in Bulgarian patients and confirmed the clinical diagnosis. Genetic consultation and prenatal diagnosis were offered to both families.

#### P13.27

#### Expression profiling of androgen and insulin pathway regulating genes unveils SOS 1 as candidate gene for idiopathic hirsutism

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Hirsutism (IH) is defined as the presence of terminal hairs in females in a male-like pattern affecting about 5% -15% of women. Hirsutism results from the interaction between the androgen level and the sensitivity of the hair follicle to the androgen. The pathophysiology of IH is presumed to be associated to SRD5A activity and generally related to an alteration of androgen receptor function. IH can lead to the metabolic syndrome, visceral obesity, dyslipidemia, insulin resistance, and hypertension.

With the aim to identify genes involved in the pathogenesis of this disorder, we investigated the expression profile of 190 genes involved both in the androgen biosynthesis and metabolism, and other genes coding for products active in the insulin pathway. The analysis was done in skin genital fibroblasts of 5 idiopathic hirsute women and 2 related controls. The array gene signature in the hirsute patients identified 4 differentially expressed genes, 2 up-regulated and 2 down-regulated ( $FC \geq \pm 1.5$ ). Differentially expressed genes included products involved in the insulin signalling while no alteration of expression level was found altered for androgens related genes. Specifically one of the over expressed transcript, coding for the SOS1 gene product, was overproduced in patients compared to controls. After sequencing the complete SOS1 gene, its promoter region and the 3'UTR region, we identified 18 SNPs potentially affecting the binding of transcription factors and the correct splicing of the gene. These findings propose

a role for SOS1, in the pathogenesis of IH, in cooperation with other insulin pathway genes.

#### P13.28

#### Bulgarian metabolomic approach for diagnosis of Inherited Organic Acidurias

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Organic acidurias are clinically important heterogeneous group of rare inherited disorders with total frequency about 1:5-6 000 newborns. Because of the unspecific clinic the diagnosis requires the application of many highly specialized methods and comprehensive approach. Metabolomic approach (extensive quality investigation of the metabolome) is the most widely used approach for this purpose.

In the National Genetic Laboratory a metabolomic approach for diagnosis of inherited organic acidurias was developed and introduced. The approach includes the next stages: urine qualitative target urine analysis of some metabolites, GC/MS urine organic acid profile, plasma amino acids and DNA analysis.

The main analytical techniques are High-effective liquid chromatography (HPLC) and Gas chromatography - mass spectrometry (GC / MS).

More than 1500 high risk patients were investigated by metabolomic approach. A total of 126 patients (8.8%) were diagnosed and classified into eleven basic organic acid disorder groups: defects of the aromatic aminoacid metabolism -13; propionate and methylmalonate metabolism - 8; branched chain aminoacid metabolism - 13; mitochondrial fatty acid oxidation - 14; pyrimidine metabolism -1;  $\gamma$ - glutamyl cycle - 2; dibasic aminoacid metabolism - 2; lactic aciduria -38; glycolysis and Krebs cycle - 8; urea cycle - 21; miscellaneous disorders - 5;. The diagnostically informative metabolite profiles are detected for each disease.

Our results can be assessed as a reasonably good and completely comparable to the data of the leading European genetic centers.

Results indicate that the application of a metabolomic approach is a useful and reliable algorithm for diagnosis of inherited metabolic diseases.

#### P13.29

#### Phenotype of LAT2 knockout mice

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LAT2 (SLC7A8) is a polytopic membrane protein that after covalent association with 4F2hc (or CD98, SLC3A2) forms an heterodimeric amino acid transporter. LAT2-4F2hc induces system L transport activity in the plasma membrane of most of the epithelial tissues (kidney, intestine, brain...). LAT2-4F2hc plays a role in the vectorial *trans*-epithelial flux of neutral amino acid (re)absorption, particularly important for cystine.

To further study the physiological relevance of LAT2 *in vivo*, we generated a knockout mice model from ES cells. Mice model deficient in LAT2 presents around 85% of lethality (n=200) despite there are no differences in weight and basic behavior compared to the wild type animals (studied up to 8 months of age).

We also generated an antibody that allows us to check the absence of LAT2 in the majority of the tissues where it is expressed. We also confirmed the basolateral location of LAT2 in the epithelial cells by immunohistochemistry with the same antibody.

In addition to these preliminary results, we are analyzing amino acid content in urine and plasma, examining the histopathology of all tissues and evaluating different behavior parameters in the knockout mice. All these studies aim to increase our understanding about the physiological role of LAT2 and its functional cooperation with TAT1 and LAT1-4F2hc, both transporters implicated in the (re)absorption in the kidney and intestine.

The LAT2 knockout mice model is viable so we have generated a potential tool to improve our knowledge of LAT2 function *in vivo* and its role in trans-epithelial flux of amino acids.

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#### P13.30

#### Progressive myoclonic epilepsy as an adult-onset manifestation of Leigh syndrome due to the m.14487T>C mutation in ND6

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Mitochondrial disorders of the oxidative phosphorylation (OXPHOS) system affect ~1/5000 individuals in the general population and present with a surprisingly wide range of multisystemic and neuromuscular phenotypes. The m.14487T>C mutation is a known pathogenic mtDNA mutation resulting in an amino acid substitution (p.M63V) in NADH dehydrogenase 6 (MT ND6), a complex I subunit of the mitochondrial respiratory chain. Thus far it has been found in isolated cases with infantile Leigh syndrome and progressive dystonia. We report here adult and late-onset phenotypes as it was seen in a 5-generation Belgian family with 12 affected family members. Clinical and mutation load data were available for 9 family members, while biochemical analysis of the respiratory chain was performed in 3 muscle biopsies. Heteroplasmic m.14487T>C levels (36-52 % in leukocytes, 97-99 % in muscle) were found in patients with progressive myoclonic epilepsy (PME) and dystonia or progressive hypokinetic-rigid syndrome. Patients with infantile LS were homoplasmic (99-100 % in leukocytes, 100 % in muscle). We found lower mutation loads (8-35 % in blood) in adult patients with clinical features including migraine with aura, Leber Hereditary Optic Neuropathy (LHON), sensorineural hearing loss and Diabetes Mellitus type 2. Despite homoplasmic mutation loads, complex I catalytic activity was only moderately decreased in muscle tissue of these patients.

**Conclusions:** The m.14487T>C mutation resulted in a broad spectrum of phenotypes in our family. This is the first report of PME as an important neurological manifestation of an isolated mitochondrial complex I defect.

#### P13.31

#### Laboratory approach for biochemical diagnosis of Lysosomal Storage Diseases

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Lysosomal storage diseases (LSD) comprise a group of more than 40 rare diseases, most of them resulting from a specific enzyme deficiency. The diagnosis is complicated due to their clinical, biochemical and genetic heterogeneity. Definitive diagnosis, as well as prenatal diagnosis of LSD are based on a specific enzyme or DNA analysis. Quantitative and qualitative analysis of urinary metabolites facilitates differential diagnosis, pointed to the probable enzyme deficiency. Appropriate scheme of assays depending on observed clinical features has been developed in our laboratory, in order to detect rapidly and reliably 27 of known LSD and to offer a prenatal diagnosis for them. In 30 years period more than 3000 patients, suspected of having LSD have been tested according to a flowchart for biochemical diagnosis of LSD. In 112 patients a pathological excretion of glycosaminoglycans have been found using quantitative dimethylmethylenblue test and qualitative thin layer chromatography or electrophoresis. Enzyme assays according to abnormal GAG patterns have been performed and 58 different mucopolysaccharidoses have been diagnosed.

Thin layer chromatography of oligocassharides has been applied to the patients with clinical features similar to MPS but with normal GAG excretion. Abnormal bands have been found in 33 cases and relevant enzyme deficiency has been demonstrated.

The definitive diagnosis on enzyme level has been set in 213 cases. Prenatal diagnosis has been undertaken in 53 cases in families with

enzymatically confirmed diagnosis in the proband.

The diagnostic rate obtained 6.8% shows unabiguosly the efficiency of the used diagnostic algorithm.

### P13.32

#### Mutation analysis of ATP7A, ATP7B and ATOX1 genes in patients with Menkes and Wilson diseases in Czech Republic

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Copper plays an essential role as a cofactor for many enzymes. There are two intracellular copper transferring P-ATPases in human: ATP7A and ATP7B, and a chaperone ATOX1 which delivers copper to them. Deficiency of ATP7A causes X-linked Menkes disease (MD). A defect in ATP7B causes autosomally recessive inherited Wilson disease (WD). Here we report the mutational analysis of the ATP7A and ATP7B genes of 4 patients with MD and 125 patients with WD from the Czech Republic.

Genomic DNA was used to amplify 23 exons of the ATP7A gene and 21 exons of the ATP7B gene. PCR products were examined by RFLP and sequenced. We introduced fast mutation screening based on differences in melting temperature of DNA fragments with sequence variations. We performed mutation analysis of the ATOX1 gene in patients whose clinical and biochemical phenotypes suggest impaired copper transport, but no mutations were found within the ATP7A and ATP7B genes.

Molecular analysis revealed 4 mutations in the ATP7A gene, two of which have not been previously published (Q724X and E1249X). 13 mutations were found in the ATP7B gene (including prevalent H1069Q mutation), and no mutations in ATOX1 gene.

Molecular analysis of the ATP7A gene allows for genetic counselling in families affected by MD. Screening for the prevalent H1069Q mutation in the ATP7B gene shows that the frequency- 44% of analysed alleles is in accordance with its occurrence in Central Europe.

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### P13.33

#### The Clinical Spectrum of Methylmalonic Aciduria Secondary to Cobalamin B Deficiency in Three Saudi Families

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We report eight patients with Methylmalonic aciduria secondary to Cobalamin B deficiency from three Saudi families. Their age ranged between four to nineteen years. The diagnosis was confirmed by vitamin B12 complementation study done on cultured skin fibroblasts. Only four of them had their first symptoms within the first few days of their life. However, all had become symptomatic by the end of their first year. All the affected children were subjected to a restricted protein diet, special formula and L-Carnitine supplement. Multiple hospital admissions per year were required for acute metabolic decompensation by the majority. Three patients died at six months, four and fourteen years of age as a result of their underlying disease. Three had developed a renal insufficiency, and one had required hemodialysis. Five children were able to attend regular schools with variable performances. One of the surviving children had sustained a severe psychomotor retardation. A detail clinical course and outcome for seven of the affected children and review of the literature is provided.

### P13.34

#### mitoNET - German Network for mitochondrial disorders

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<sup>5</sup>Neurologie, Charité Virchow Klinikum, Berlin, Germany, <sup>6</sup>Technical University of Munich, Stoffwechselzentrum Kinderklinik, München, Germany, <sup>7</sup>University Bonn, Department of Epileptology and University Jena, Bonn, Germany,

<sup>7</sup>Friedrich-Baur-Institute, Dept. of Neurology Ludwig-Maximilians-University of Munich, München, Germany, <sup>8</sup>Klinikum der Stadt Ludwigshafen gGmbH, Institute for Clinical Chemistry and Molecular Diagnostics, Ludwigshafen, Germany. Aims: The principal goal of mitoNET is to establish a network of clinical and basic scientists in order to improve health care for patients with mitochondrial diseases.

Work plan Steps to achieve this are:

- Buildup of a nationwide network of neurological and paediatric departments for recruitment and phenotyping of patients, setup of a web-based register, and conduction of longitudinal studies;
- Comprehensive collection and storage of biological materials including DNA, RNA and myoblasts, providing aliquots to researchers within and outside the net;
- Enhancing the range of diagnostic tools, including new assays to quantify mitochondrial proteins and dynamics, high-throughput genotyping, and a systems approach;
- in vitro* investigations of novel treatments including an approach to improve respiratory chain deficiency by fibrate-induction of peroxisome proliferator-activated receptor pathways and an approach to identify mechanisms behind and strategies against propagation of mtDNA mutations;
- Increased collaboration of basic and clinical researchers to boost synergy effects, interdisciplinary cooperation and training initiatives;
- Increased public and professional awareness.

Expected results: Progress in the projects of the consortium and the additional boost for collaboration, synergy and communication will lead to an improvement in diagnostics, therapy and medical care for patients with mitochondrial diseases.

### P13.35

#### Respiratory pathology in monozygotic sisters with MPS1

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Mucopolysaccharidosis 1 (MPS1, Hurler syndrome, MIM252800) is an autosomal recessive disease characterized by variable systemic manifestations, increased urinary mucopolysaccharide excretion and the defective  $\alpha$ -L-iduronidase (IDUA, EC 3.2.1.76). The MPS1 gene has been mapped to chromosome band 4p16.3. Two common nonsense mutations W402X and Q70X are responsible for between 15 and 65% of mutant alleles depending on the population. We report on 8-year-old monozygotic sisters with MPS1 caused by IDUA deficiency (genotype Q70X/Q70X). The probands have severe variant of MPS1, including coarse facial features, corneal opacity, skeletal dysplasia, dysostosis multiplex, mental deficiency and hepatosplenomegaly. They suffer from frequent attacks of respiratory lung disorders. Both sisters get rhinitis, acute bronchitis three or four times each year. One of the monozygotic sisters got bronchopneumonia last year. Both girls had the sings of emphysema and fibrosis of lung tissue revealed with X-ray observation. We suppose that pulmonary disorders in our children are caused by inborn error of mucopolysaccharide degradation because a deficiency of the lysosomal enzyme IDUA results in accumulation of the mucopolysaccharides dermatan sulfate and heparin sulfate throughout tissues and organs of our patients. Pessimistic prognosis, death from MPS1, is usually caused by upper airway obstruction and pulmonary complications. We use enzyme replacement therapy with iduronidase. Although the phenotypes were slightly modified there was still physical handicap and developmental delay.

### P13.36

#### Age-dependent changes of the mitochondrial DNA content in muscle, liver and fibroblasts

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Tissue-specific depletion of mitochondrial DNA (mtDNA), caused by mutations in increasing number of genes, often gives rise to serious mitochondrial disorders. Recently, a real-time PCR (qRT-PCR) has

been widely used to determine mtDNA content. Nevertheless, this high sensitive method requires a large number of tissue-specific and age-matched controls. To bypass the lack of controls, we analyzed the mtDNA amount in available samples from patients referred to our laboratory to exclude mitochondrial disease. Specimens with decreased relative amount of mtDNA compare to other age-matched samples were selected for mutation screening of candidate genes.

We quantified mtDNA amount by the qRT-PCR as described previously (Pejznochova, 2008) in almost 200 muscles, 50 liver and 80 cultured-skin fibroblasts specimens. MtDNA content was expressed as a copy-number ratio of mtDNA to nuclear DNA. Samples with confirmed deletion or point mutation in mtDNA were excluded from the analysis. The relative amount of mtDNA was found to gradually increase during the first 20 years in both muscle ( $n=98$ ,  $r=0.7$ ,  $p<0.01$ ) and liver ( $r=0.5$ ,  $p<0.01$ ) while in fibroblasts it appears to remain largely unchanged. Besides, four samples with decreased mtDNA content were selected for consecutive mutation analysis of *POLG* or *TK2* genes.

Our results are in accordance with previously published data in disease-free samples and accentuate the age dependence of mtDNA amount evaluation. Moreover, our data can be temporary utilized in a candidate searching for a sequencing of mtDNA depletion-related genes.

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### P13.37

#### Mucolipidosis type II

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Mucolipidosis II (or I-cell disease) (ML II) is a rare autosomal recessive lysosomal storage disease due to N-acetylglucosaminyl 1-phosphotransferase deficiency (EC 2.7.8.15). This phosphotransferase catalyzes the initial step in the synthesis of the mannose 6-phosphate determinant required for efficient intracellular targeting of newly synthesized lysosomal hydrolases to the lysosome. This enzyme is encoded by 2 different genes. The altered gene coding for the a/b sub-units is localized on chromosome 12p.

Clinical and radiological signs are similar to those in Hurler's disease, although onset is during the first months of life. It is characterized by hypertrophic gingivae, enlarged tongue, coarse facies, hirsutism, hernias, cutaneous infiltration, limited joint mobility, dysostosis multiplex, hepatosplenomegaly, corneal opacities, deafness, mental and motor retardation, and dwarfism. The authors present a boy diagnosed with mucolipidosis type II at the age of four years..

### P13.38

#### Mucopolysaccharidosis I: One More Attempt to Evaluate Efficacy of Enzyme Replacement Therapy.

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Background: Mucopolysaccharidosis I (MPS I) is a progressive disease caused by the deficiency of alpha-L-iduronidase due to mutations in 4p16.3. Clinical trials (ALID-014-02, BIO7500-001, ALID-003-99, ALID-006-01, ALID-017-03) showed the recombinant human enzyme (Aldurazyme®, Genzyme) replacement therapy (ERT) to be enough safe and effective.

Objectives: Evaluation of the safety and efficacy of ERT with Aldurazyme® in Russian children with MPS I.

Methods: Non-randomized, open label, uncontrolled, single group assignment study with eligibility criteria similar to protocol ALID-014-02 but without limitations on age. Two girls (7 years) and a boy (7 years) with Hurler syndrome were enrolled. Patients received intravenously Aldurazyme® at a weekly dose of 100 U/kg for 84 (boy) and 56 weeks (girls). Criteria of evaluation: urinary glycosaminoglycan excretion (GAGe), vital signs, the investigator's global assessment.

Results: The GAGe decreased 56%, 33% in the girls, 71% in the boy, the reduction being associated with clinical benefit in the girls. In the girls a decrease in liver and spleen size was evident. All children had mild heart left ventricular hypertrophy, and hydrocephaly which didn't progress. Improvement in psychomotor, emotional status was noticeable. Anxiety, fever, urticaria after several infusions were considered

to be mild adverse effects and were completely eliminated with acetaminophen.

Conclusions: Though the decline in GAGe was close to that for ALID-014-02 trial and the duration of treatment was even longer, the efficacy was evident, but not so prominent. Measurements of Health Related Quality of Life will help to evaluate the evidence-based efficacy of ERT in MPS I children.

### P13.39

#### Incidence of the Mucopolysaccharidoses in Taiwan, 1984-2004

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Previous studies on the incidence of MPS diseases in different populations have shown considerable variation. However, information regarding the incidence of MPS in Asian population is lacking. An epidemiological study of MPS in Taiwan using multiple ascertainment sources was undertaken, and incidences of different types of MPS during the period of 1984 to 2004 were estimated. We compared our data with previous reports in different populations. The combined birth incidence for all MPS cases was 2.04 per 100,000 live births. MPS II had the highest calculated birth incidence of 1.07 per 100,000 live births (2.05 per 100,000 male live births), comprising 52% of all MPS cases diagnosed. The birth incidences of MPS I, III, IV, and VI were 0.11, 0.39, 0.33, and 0.14 per 100,000 live births, respectively, which accounted for 6%, 19%, 16%, and 7% of all MPS, respectively. No cases of MPS IIID, IVB, VII or IX were ascertained during the study period. Overall incidence of MPS diseases in Taiwan was consistent with that reported in Western populations. In contrast to the higher incidence of MPS I in most Western populations, this study showed a higher incidence of MPS II in Taiwan. It remains to be investigated whether this discrepancy is attributed to the underdiagnosis of MPS I in Taiwan or to ethnic differences.

### P13.40

#### Mucopolysaccharidosis in IRAN (A fifteen years survey)

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More than 500 different phenotypes of metabolic disorders identified and categorized in the medical literature during 20<sup>th</sup> century after Garrod. Now for the most of them biochemical and molecular background has been identified. Individually they are rare disorders but as a whole 1 of every 1000 live-borne babies may suffer from one of these diseases. Consanguinity is a predisposing factor, because in the most of them inheritance pattern is autosomal recessive. Between them mucopolysaccharidoses are common rare disorders with characteristic phenotypes. Clinically they are chronic, progressive, and disabling disorders.

Because the rate of consanguineous marriages are very high in our population, these diseases are prevalent and now they constitute a very important health problem in our community.

We have studied 134 families with 278 cases in the past 15 years diagnosed as MPS. The diagnosis in the index cases (after a detailed clinical, biochemical, radiological, imaging, and.....) confirmed by enzyme assay in the genetic and metabolic department of the Erasmus university and the Centogene laboratory in the Rostock. Prevalence of different types have been shown in the table. The most common type was MPSIII, followed by MPSII and MPSI respectively. Prenatal diagnostic tests carried out in 89 pregnancies and 27(33%) affected fetuses detected.

Normal	Affected	PND	No of cases	No of families	Diagnosis
			87	40	MSP III
			45	16	MSP III A
			25	14	MSP III B
			2	3	MSP III C
			14	6	MSP III D
			70	29	MPS II
			52	28	MPS I
			33	17	MPS VI
			33	17	MPS IV
62	27 (33%)	89	278	134	Total

Relative incidence of MPS cases &  
Prenatal Diagnosis in affected Iranian families

#### P13.41

##### Functional analysis of the gene deficient in MPS IIIC patients

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Mucopolysaccharidosis (MPS) IIIC is an autosomal recessive lysosomal storage disorder caused by a deficiency in heparan acetyl CoA: alpha-glucosaminide N-acetyltransferase (HGSNAT). This is localised to the lysosomal membrane and catalyses a transmembrane acetylation in which the terminal glucosamine residue of heparan sulphate acquires an acetyl group, thus forming *N*-acetylglucosamine. The characteristic feature is the deterioration of the central nervous system, but other symptoms may include coarse facies, developmental delay, macrocrania and motor retardation. Only recently has the gene for MPS IIIC been identified. *HGSNAT* is localised to chromosome 8p11.1 and contains 18 exons. The cDNA codes for a product of 635 amino acids which is predicted to contain a cleavable signal peptide at its N-terminus, 11 transmembrane domains, and up to 5 N-linked glycosylation sites. We and other groups have since sequenced 45 different disease-causing *HGSNAT* alleles in MPS IIIC patients. 19 of these are missense mutations, although these are yet to be functionally analysed. Furthermore, certain biochemical properties of HGSNAT, such as its active site, remain uncharacterised. In this study, a number of mutations known to occur in MPS IIIC patients have been introduced into the cDNA of *HGSNAT*. The expression of these *HGSNAT* derivatives in cell culture and the analysis of their protein levels and activity have been performed to examine the effect of the tested mutations on HGSNAT function. This research will aid in detailing the biochemical characteristics of HGSNAT activity, and thus the molecular basis of MPS IIIC.

#### P13.42

##### GBA gene variants are associated with PD in France

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##### Objective :

To compare the frequency of variants in the *GBA* gene which encodes  $\beta$ -glucocerebrosidase, in Parkinson's disease (PD) patients and in controls. To compare the clinical features of GBA-PD patients with PD patients without any mutations.

##### Background :

Homozygous mutations of *GBA* are responsible for Gaucher disease. It has been reported that heterozygous mutations of *GBA* was a risk factor for PD, especially in Ashkenazi Jews.

##### Methods :

*GBA* sequencing was performed in 293 mostly (87%) French PD pa-

tients and 252 age-matched controls.

##### Results :

Mean age at examination was  $57.7 \pm 11.5$  years (33-85) for PD and  $57.8 \pm 11.9$  (31-85) for controls. Mean age at onset and mean disease duration were  $47.5 \pm 10.0$  years (30-70) and  $10.3 \pm 6.6$  (1-30). *GBA* variants were more frequent in PD compared to controls (11% versus 0.4%,  $p < 0.0001$ ). We detected the following variants which are considered of severe (S), intermediate (I) or unknown (U) severity: N370S (I, n=7), L444P (S, n=2), D409H (S, n=1), R131C (S, n=1), E10V (U, n=2), Y212N (U, n=2), D282N (U, n=1), A292P (U, n=1), the combination of E326K (homozygous) + D410H (S, n=1), the combination of L444P + A456P + V460V (S, n=1) and the following SNP: E326K (modifier, n=11), T369M (neutral, n=3). Four variants (U) were newly described. GBA-PD had significantly delayed onset of treatment-induced fluctuations ( $186 \pm 229$  versus  $52 \pm 42$  months,  $p = 0.013$ ) and more often increased lower limbs reflexes (25% versus 10%,  $p = 0.026$ ).

##### Conclusion :

*GBA* heterozygous variants are significantly associated with PD in the French population and influence the phenotype.

#### P13.43

##### The role of Methionine metabolism in the clinical manifestations of Phenylketonuria

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Phenylketonuria (PKU) is an inborn poly-enzymatic multi-system pathology of the metabolism, its primary block being the Phenylalanine (Phe) hydroxylation that leads to severe mental retardation with clinical and biochemical polymorphism.

**Methods:** 30 PKU children were investigated for the level of free amino acids in blood and urine determined through liquid chromatography on amino acid analyzer Kovo AAA339, Czech. They presented the following troubles: excitability (97%), capricious mood (93%), mental retardation (47%), hyperactivity (53%), tics, convulsions (13%), deficiency of attention and memory (80%), sleep disturbance (83%).

**Results:** In addition to the Phenylalanine transformations troubles, we appreciated the increased levels in blood of: Cysteine (by 2.5 times,  $p < 0.001$ ), cysteinic acid (by 4 times,  $p < 0.05$ ), cystathione (by 1.3 times), Homocysteine and low levels of: Methionine (by 1.8 times,  $p < 0.05$ ) and Taurine (by 1.2 times). The urine amino acids spectrum was following: the high level of cysteinic acid (by 1.4 times), cystathione (by 3.8 times,  $p < 0.05$ ), Homocysteine, Taurine (by 1.6 times) and low level of Cysteine (by 1.6 times,  $p < 0.001$ ).

We added to the traditional PKU diet the drug metabolic correction containing the vitamins of group B (50-150 mg/day) and Taurine (250-500 mg/day). As the result, the children showed calmer and equilibrator, the hyperactivity and seizures decreased, sleep disturbance disappeared, the attention and memory increased, the cognitive capacity improved.

**Conclusion:** The effectiveness of the PKU treatment increases if combining the low Phe diet with drug metabolic correction, considering all metabolic components. We consider, that will be better if the PKU formula be enriched with high doses of vitamins B and Taurine.

#### P13.44

##### Genetic and biochemical characterisation of a novel splice-site mutation in mitochondrial polymerase gamma (POLG1)

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**Aim:** Genetic and biochemical investigations in a 3y old boy suspected for *POLG1* defect due to valproate induced fatal cholestatic hepatopathy.

**Methods:** Patient's DNA was analysed for *POLG1* mutations and consequences of the splice site mutation was analysed by reverse transcription. The content of mtDNA of patients liver, fibroblast and muscle biopsies was quantified by qRT-PCR. The activities of respiratory chain complexes (RCC) were measured spectrophotometrically in various

tissues. BN-PAGE from liver and muscle biopsies was performed, followed by in gel activity assays.

**Results:** Sequence analysis of POLG1 gene revealed compound heterozygosity for the most commonly found mutation p.A467T in exon 7 and a novel splice-site mutation c.1251-2A>T in intron 6 resulting in an in frame skipping of Exon 7. The liver biopsy showed marked deficiency of Complex I, III and IV. In muscle we found an isolated Complex IV deficiency, whereas in fibroblasts all activities were normal. There was a 90% mtDNA depletion in liver, no depletion in muscle and a 30% depletion in fibroblasts. In liver there was a reduced in gel activity for complex I, no activity for complex IV, the activities of complex II were equal to the control. In muscle we found decreased activity of complexes I and II and no activity for complex IV.

**Conclusion:** This new splice site mutation affects a highly conserved region of the protein and leads in combination with p.A467T to different depletion levels in different tissues. It extends the number of POLG1 mutations associated with fatal childhood hepatocerebralopathy.

#### P13.45

##### Novel *SUCLG1* mutations in a patient with encephalomyopathic phenotype associated with mild methylmalonic aciduria and mtDNA depletion

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The mitochondrial DNA depletion syndromes (MDS) are a heterogeneous group of severe mitochondrial disorders inherited as autosomal recessive traits. Three main clinical forms have been described: myopathic, encephalomyopathic and hepatocerebral. Recently, the *SUCLA2* and *SUCLG1* genes, which code for different subunits of succinate-CoA ligase, have been involved in patients with severe encephalomyopathy and mild methylmalonic aciduria. However to date, only one family with *SUCLG1* mutation has been reported.

Herein, we report the clinical and molecular findings in a child with encephalomyopathic MDS secondary to novel *SUCLG1* mutations.

At birth, this child presented with a severe hypotonia, respiratory failure and hypoglycemia. Metabolic investigations revealed lactic acidosis and mild methylmalonic aciduria. Histologic, biochemical and molecular analyses of the muscle showed respectively cox-negative fibers, combined respiratory-chain enzyme deficiency and mtDNA depletion. We identified two novel mutations in *SUCLG1*. One allele, inherited from the mother, carried a missense mutation that changes a highly conserved C to G (c.509C>G, p.Pro170Arg). Amino acid conservation, *in silico* predictions and absence of this variant in 160 controls are strong arguments for its pathogenicity. The second allele, inherited from the father, carried a G to C substitution (c.97+3G>C) in intron 1. *In silico* studies predicted this variant to affect splicing. Functional analyses using reporter minigenes are in progress to ascertain the splicing outcome of this intronic variation.

We described the clinical evolution of a child with MDS due to *SUCLG1* mutations confirming its role in encephalomyopathic MDS with mild methylmalonic aciduria.

#### P13.46

##### *TCF7L2* polymorphism is an independent risk factor for New Onset Diabetes Mellitus After Transplantation: a cohort study on 1229 renal transplant patients.

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**Objective:** Whether New Onset Diabetes Mellitus After Transplantation (NODAT) shares the same susceptibility genes with type 2 diabetes mellitus has not been adequately assessed to date. The aim of our study was to investigate the association between 11 type 2 diabetes mellitus-associated polymorphisms and the risk of NODAT within the first 6 months after-renal transplantation.

**Methods:** A total of 1229 patients free of diabetes at transplantation were genotyped for 11 polymorphisms: rs7903146 (*TCF7L2*),

rs8050136 (*FTO*), rs7754840 (*CDKAL1*), rs5215 (*KCNJ11*), rs1801282 (*PPARG*), rs1111875 (*HHEX-IDE*), rs13266634 (*SLC30A8*), rs10811661 (*CDKN2A-CDKN2B*), rs4402960 (*IGF2BP2*), rs757210 (*HNF1B*), rs10010131 (*WFS1*). NODAT was defined by fasting plasma glucose 126 mg/dL on at least two occasions or *de novo* hypoglycemic therapy.

**Results:** Patients who developed NODAT (N=145, incidence=11.8%) within the first 6-months post-transplantation were compared to patients free of NODAT (N=1084) for clinical and genetic factors. NODAT was significantly associated with the following characteristics by multivariate analysis: *TCF7L2* polymorphism (P=0.014), older age (P<0.0001), black African or north-African ethnicities (P=0.003), higher body mass index at transplantation (P=0.016), tacrolimus (P=0.01) and mTOR inhibitors (P=0.003). The risk to develop NODAT was 1.55 (OR; 95%CI: 1.06-2.25; P=0.02) for CT genotype and 1.79 (OR; 95%CI: 1.02-3.14; P=0.04) for TT genotype, in comparison with the CC genotype of rs7903146. No other polymorphism was significantly associated with NODAT.

**Conclusions:** Our results show the independent contribution of the *TCF7L2* polymorphism in NODAT, and suggest a common insulin secretion defect pathway with type 2 diabetes mellitus. Our results may help to tailor immunosuppression in order to prevent NODAT.

#### P13.47

##### Identification of the tMTHFR mutation in a case with portal cavernoma

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**Introduction.** Homocysteine is an amino acid important in metabolism. Most folate-responsive mild homocystinemia is due to homozygosity for a common C677T polymorphism in methylene tetrahydrofolate reductase that produces a thermolabile enzyme (tMTHFR). Approximately 10% of whites are homozygous for tMTHFR. As compared to the general population, persons with hyperhomocystinemia have a 6.8 time greater risk of peripheral arterial disease, 2.5 time greater risk of stroke, and 2 time greater risk of heart disease.

**Case presentation.** A 5 year old female was admitted for asymptomatic thrombocytopenia and leucopenia discovered to an occasionally laboratory exploration. Her grandfather died due to mesenteric thrombosis and the patient presented in the neonatal period septicemia with left temporo-mandibular arthritis that recovered with ankylosis and left mandibular hypoplasia. Clinical exam revealed splenomegaly without hepatomegaly. Laboratory tests confirmed thrombocytopenia (41.000/mmc) with leucopenia (2300/mmc) and bone marrow aspirate analysis excluded a hematological disease. Abdominal Eco Doppler revealed the absence of flux in portal vein and portal cavernoma was confirmed by angio-MRI. AT III, C protein, S protein were in normal range but homocysteine level was increased. Genetic analysis detected homozygous C677T mutation in tMTHFR. We consider that the genetic confirmation is crucial for the therapeutic measures, not only in folic acid supplementation but mainly in thrombosis prophylaxis that is obligatory in all surgery interventions as in all known situations that increase the thromboembolic risk.

#### P13.48

##### Familial investigation of a rare mitochondrial myopathy

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The vast majority of mitochondrial mutations affect tRNA genes. Mutations in different tRNA genes and different mutations within a same tRNA can cause distinct clinical and biochemical phenotypes with variable expression and age of onset. This renders genetic counseling very difficult.

We report on a rare A3302G tRNA<sup>Leu(UUR)</sup> mutation in 5 family members. The proband developed at 2 years of age progressive axial and limb girdle myopathy. She subsequently lost head control due to severe axial hypotonia. At present, she is wheel-chair bound and suffers from frequent bronchitis with respiratory decompensation. Biochemical analyses demonstrated a deficit in the complex I of the respiratory chain in the muscle biopsy. Genetic analyses showed a 95% mutant load

in the muscle, 91% in epithelial cells and 97% in lymphocytes. Family investigation revealed that the grandmother, mother, aunt and uncle of the proband were all carriers with a mean mutant load of 10%, 40%, 5% and 20% respectively. The heteroplasmy was constant across tissues. These four relatives are asymptomatic and despite relatively high mutant load (i.e.: 40% in the mother), biochemical analysis revealed normal complex I activity in the muscle biopsy performed in the mother and her two siblings.

These results provide important insight on genotype-phenotype correlation. Positive mutant load observed in an older (70y.) asymptomatic individual as well as normal OXPHOS assays in the other unaffected adults demonstrate that high mutant loads are probably necessary for symptoms to develop. These findings as well as heteroplasmy consistency through tissue type could represent valuable information for family members seeking accurate genetic counseling or prenatal testing.

#### P13.49

##### Spectrum of Wilson Disease Gene (ATP7B) and ATOX1 Mutations in an Isolated Romanian Population

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Wilson disease is an autosomal recessive disorder characterized by dramatic build-up of intracellular hepatic and brain copper. WD is caused by mutations in the gene ATP7B. We report the further results of an ongoing project concerning the spectrum of mutations on the ATP7B gene patients from an isolated Romanian population with high prevalence of WD. Direct sequencing of all 21 exons within ATP7B gene revealed that four WD patients are heterozygotes or compound heterozygotes bearing three previously reported mutations: P767P-fs, H1069G and K832R (considered by some researches a polymorphism). We found that one WD patient has only K832R polymorphism in homozygosity. His parents are homozygote for K832R and heterozygote for P767P-fs. The boy has all WD manifestation associated with a mild disruption of copper metabolism based only on the presence of hepatic disturbance and we supposed the presence of two disease-causing mutations in his DNA. Proteins interacting with the ATP7B copper transporter such as ATOX1 are important in explaining this phenomenon. Human ATOX1 protein regulate the catalytic activity of ATP7B protein by binding and transporting cytosolic copper to ATPase proteins in the trans-Golgi network for later incorporation to the ceruloplasmin. Mutation analysis of the four exons of the ATOX1 gene was performed in all five WD patients diagnosed by DNA analysis. Direct sequencing of the ATOX1 gene within the 5'-UTR region revealed one known heterozygous polymorphism (T/C at 5'UTR -99) in one Wilson patients. The genetic base of Wilson Disease in our K832R WD patient remains unidentified.

#### P14.01

##### Induction of $\gamma$ -globin by knockdown of MBD2 and C1C2 in K562 cells by siRNA

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At present a number of chemicals that induce expression of the fetal  $\gamma$ -globin gene are used as treatment for  $\beta$ -thalassemia. One of these chemicals is a DNA methyl transferase inhibitor called 5-Azacytidine (5-Aza). A potential downstream effector ( $\gamma$ -globin repressor) of 5-Aza is a protein called Methyl Binding Domain protein 2 (MBD2). We sought to determine whether knockdown of MBD2 by siRNAs in human erythroid cells would result in induction of  $\gamma$ -globin mRNA. This information could be used to develop more targeted  $\beta$ -thalassemia drugs. To this end, an MBD2-specific validated siRNA was transfected into K562 erythroleukemia cells and the levels of MBD2 and  $\gamma$ -globin compared to cells transfected with a scrambled siRNA control by using qRT- PCR. MBD2 levels were knocked down to  $0.65 \pm 0.23$  and  $\gamma$ -globin levels were increased to  $1.70 \pm 0.46$ . In contrast, the largest  $\gamma$ -globin

mRNA increase obtained after exposure of K562 cells to 5-Azacytidine was 7.36 fold—seen after a 3 day exposure to 15  $\mu$ M of 5-Aza. These results suggest that MBD2 siRNAs can indeed induce  $\gamma$ -globin at significant levels. The DNA binding site of MBD2 is not clear. We sought to determine whether MBD2 was mediating its repressive activity via LARC. To this end siRNAs against C1C2 shRNP, the DNA binding protein of LARC, were transfected into K562 cells and C1C2 and  $\gamma$ -globin levels measured as discussed above. C1C2 levels were decreased to  $0.54 \pm 0.16$ , whereas levels of  $\gamma$ -globin remained unchanged ( $1.07 \pm 0.25$ ). These results suggest that the repressive effect of MBD2 is not mediated through its binding to LARC.

#### P14. Therapy for genetic disorders

##### P14.02

##### Breast cancer prevention by letrozole in post menopausal BRCA1/2 mutations carriers : the onco-03/LIBER trial

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Women carrying germline BRCA1/2 deleterious mutations represent an extreme risk population for developing breast cancer, with a cumulative life-time risk of 56-80%. Although it greatly affects the quality of life, breast prophylactic surgery in BRCA1/2 mutation carriers have increased in Europe and in US over the last decade. Medical prevention of breast cancer could thus provide a precious alternative to prophylactic mastectomy.

The major breast cancer prevention trials using tamoxifen and raloxifene showed an approximately 50% risk reduction in high risk women. The contralateral risk reduction in current adjuvant trials comparing aromatase inhibitors (AI) to tamoxifen reveals a higher preventive efficacy of AI after menopause. Two ongoing randomized studies using exemestane (MAP3) or anastrozole (IBIS2) are assessing the risk reduction of breast cancer but none are designed for BRCA1/2 carriers. The French federation of cancer centres, "FNCLCC" and the French national cancer institute (INCa) have developed a randomized phase III study to determine the efficacy of an aromatase inhibitor (letrozole) to reduce the incidence of invasive breast cancer in post menopausal BRCA1/2 carriers. The ONCO-03 (LIBER) study is a double-blind, letrozole versus placebo, controlled study involving 30 centres in France. The study opened for recruitment in February 2008. The study design, procedures and first analysis of patients enrolment are presented. For women bearing a BRCA1/2 genetic predisposition, evaluation of medical prevention of breast cancer risk is needed to offer an additional option to surveillance or bilateral mastectomy.

##### P14.03

##### Endothelial function and plasma thiols in patient with CADASIL (Cerebral autosomal dominant arteriopathy with subcortical infarct and leukoencephalopathy)

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CADASIL, a rare disorder due to point mutations of Notch3 gene, is characterized by recurrent strokes, serious motor disability, pseudobulbar paralysis and subcortical dementia. The mutation modifies a transmembrane receptor involved in the arterial maturation inducing structural anomalies of endothelial cells and smooth muscle of small vessels. Endothelial dysfunction (ED), an important prognostic marker in cardiovascular disorders, is associated to defective endothelial production of nitric oxide (NO); the essential cofactor of NO-synthase, BH4, increases NO bioavailability. We designed a study to assess whether endothelial function may be improved by BH4 administration in 60 CADASIL patients enrolled at 5 Italian centres. We report on

baseline data of endothelial function, assessed by measurement of changes induced by reactive hyperemia in pulsatile volume at fingertips, through a non invasive plethysmographic method (EndoPAT Itamar Israel) and redox state as determined by blood thiol concentrations. Preliminary results show that CADASIL patients have a lower endothelium-dependent vasodilatation (PAT index) than healthy controls with/without conventional cardiovascular risk factors (RF). Patient categorization according to their RF profile shows an interaction between CADASIL and the coexisting RFs: PAT index is lower in patients with  $\geq 1$  RF [ $n=19$ , 1.68 (1.44-1.92)] vs those with no RF [ $n=28$ , 2.01 (1.77-2.60)] ( $P\leq 0.03$ ). Thiol profile showed significantly increased cysteinylglycine ( $37\pm 7$  vs  $32\pm 9$   $\mu\text{mol/l}$ ,  $P=0.02$ ) and decreased glutathione ( $5.5\pm 2$  vs  $7.7\pm 3$   $\mu\text{mol/l}$ ,  $P=0.02$ ) concentrations in CADASIL patients vs healthy controls.

These findings underscore the importance of specific assessment of ED and redox state and accurate management of RF in CADASIL patients.

#### P14.04

#### The benefit of an intensive rehabilitation program in patients with cerebral palsy

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**Background:** Cerebral palsy is defined as a group of permanent disorders of the development of movement and posture, causing activity limitation, attributed to non-progressive disturbances that occurred in the developing brain. Cerebral palsy may be due to many causal factors that act during prenatal, perinatal or postnatal life, most cases seeming to be of early prenatal origin.

**Objective:** The aim of the present study was to demonstrate that application of an intensive program of physical therapy considerably improves the global motor function.

**Material and methods:** We studied a lot of 20 children with cerebral palsy (12 with pyramidal tetraparesis and 8 with pyramidal hemiparesis), mean of age 4.4 years, beneficiaries of a physical kinetic program, specific for each type. The lot was divided in two groups: control group (10 subjects) and experiment group (10 subjects). Subjects included in control group followed a rehabilitation program for 6 months, 3 times a week, whereas subjects included in experiment group followed an intensive rehabilitation program - 2 times a day, for 6 months. The global gross motor function was evaluated for each child before and after therapeutic intervention (GMFM 88).

**Results:** The gross motor function of children from both groups improved significantly after this intervention. Children from the experiment group performed better and showed significantly greater improvement than those from the control group.

**Conclusions:** Application of an intensive rehabilitation program determines increasing of motor independence in children with infantile cerebral palsy and allows creating of a favorable climate for their integration in society.

#### P14.05

#### Enzyme-Free and High-throughput cloning means for production of recombinant protein and gene therapy

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DNA cloning is one of the most utilized techniques in molecular biology research. Using the traditional DNA cloning methods, which include usage of restriction endonucleases and DNA ligase, have many limitations including unavailability of unique restriction sites in both insert and vector. LIC (Ligase-Independent Cloning) methods have been developed to overcome such problems, they use different enzymes to generate long enough "sticky ends" that can hold insert and vector together. Reparative mechanism of cell can join the nicks in both strands and final construct can be formed.

Here we describe a simple method for the cloning of PCR products without the need for restriction enzyme and any post enzymatic treatment. PCR products containing phosphorothioate bonds are used to create complementary single stranded overhangs on both insert and vector by a post-PCR iodine treatment. These single stranded overhangs are designed to allow directional cloning without using any enzyme (Enzyme-Free cloning). After hybridization and transformation

step construct can be isolated from cells. Our procedure is faster than the existing cloning strategies and also is highly efficient, directional and reliable. Besides that since two different cohesive ends are used, the orientation of the insert in relation to the vector can be controlled. Flexibility in choice of vector and insert makes this method exceptionally suitable for high-throughput cloning. This characterization is particularly useful for ensuring that a DNA fragment is directionally cloned into the correct reading frame for protein expression or for the creation of fusion proteins.

#### P14.06

#### Improvement of life quality in patients with cystic fibrosis through kinetotherapy and physical exercises

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**Background:** The physical treatment is one of the most important aspects of the management of Cystic Fibrosis. The problems of the lungs remain the major difficulty which has to be dealt with on a day-to-day basis.

The aim of study was to prove the efficiency of several physiotherapy techniques and to adapt them according to the age and adherence of patients.

**Methods:** 71 patients aged between 2 month and 18 years were followed up over the last 4 years, 2004-2008 in the National Cystic Fibrosis Centre and divided in 3 groups according to age and compliance: group I: 15 infants age between 2 months-3 years old, group II: 14 children between 3-6 years old, group III: 42 children between 6-18 years old. We have assessed: clinical status, nutritional condition, X-ray, bacteriological exam, MEF<sub>25-75%</sub>, FEV<sub>1</sub>, PEF.

**Results:** The result at the first group has indicated an improvement in all parameters. At the second group the maximum compliance was in physical exercises 86%, and the maximum efficiency 72% was in the combined techniques. The statistical briefing of data in the third group shows the fact that there are significant statistical difference ( $p< 0.05$ ), before and after treatment in all ventilator index.

**Conclusions:** The chosen technique proved to be very efficient, in improving of respiratory symptoms and ventilator parameters. It is extremely important to make sure that the treatment advised for each individual patient is that which is most suitable to them for their age, cultural background and disease severity.

#### P14.07

#### Effects of early rehabilitation in a plurimalformative syndrome due to deletions of chromosomes 13 and 18-case report

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Deletion of the long arm of chromosome 18 is a chromosomal disorder with a phenotype that may vary considerably in range and severity, depending on the type of deletion and location of the breakpoints. Children have characteristic features including short stature; mental retardation; hypotonia, malformations of the hands and feet; craniofacial abnormalities and numerous neurologic deficiencies with a high incidence of dysmyelination. Deletion of long arm of chromosome 13 is characterized by malformations of the craniofacial region, skeletal abnormalities, other physical abnormalities and intellectual disability. In this paper we report the case of a female infant with multiple congenital abnormalities, craniofacial dysmorphism, heteroataxia, severe mental retardation and severe hypotonia, who was found to have deletions of the long arm of chromosomes 13 and 18. We included her in a rehabilitation program from the age of eleven months. Rehabilitation programs aimed improving hypotonia as well as stimulating the development of motor skills. We observed the child for a period of one year, periodic monitoring of muscle tone and performance along with the neurological status showing significant motor and mental improvement. Conclusions: Rehabilitation treatment is effective and must be an early intervention.

## P14.08

**Musculoskeletal and central nervous system (CNS) response to early administration of enzyme replacement therapy (ERT) for infantile Pompe disease**M. Rohrbach<sup>1</sup>, A. Klein<sup>2</sup>, C. Balmer<sup>3</sup>, M. Baumgartner<sup>1</sup>,<sup>1</sup>Department of Metabolics, University Children's Hospital, Zurich, Switzerland,<sup>2</sup>Department of Neurology, University Children's Hospital, Zurich, Switzerland,<sup>3</sup>Department of Cardiology, University Children's Hospital, Zurich, Switzerland.

Pompe disease is a rare lysosomal glycogen storage disorder, characterized by deficiency of acid  $\alpha$ -glucosidase enzyme (GAA) caused by mutations in the GAA gene, leading to accumulation of glycogen filled lysosomes and autophagic vesicles mainly in the musculoskeletal system. Infantile type Pompe disease is a multiorgan disorder presenting with cardiomyopathy, hypotonia, respiratory insufficiency, and early death by 1 year of age.

We report a 2 8/12 year old girl with infantile Pompe's disease, on ERT started at age of 8 weeks. She presented with severe hypertrophic cardiomyopathy (left ventricular cardiac mass 132g/m<sup>2</sup>), facial, axial and proximal weakness; Pompe disease was confirmed by undetectable serum GAA activity and a homozygous mutation (c.1157insA) in the GAA gene. Brain MRI was normal as was lung function testing. ERT with 20mg/kg Myozyme® biweekly resulted in significant reduction of LVM of 50% within 12 months of treatment, and reduction of LVM on MRI to 54 g/m<sup>2</sup> at 30 months of age. Brain MRI at 18 and 30 months of age, respectively, revealed symmetrical signal alteration of the deep white matter

and no delay in myelination milestones. Motor development and muscle power was almost normal at 30 months, however cognitive development was markedly delayed especially the speech development, unexplained by mild sensoneuronal hearing impairment.

While musculoskeletal and cardiac tissue responded well to ERT, CNS manifestations are persisting. More data is needed to evaluate whether longer survival by ERT unmasks the CNS phenotype and to better understand the limitations of ERT itself.

## P14.09

**Cell survival suppression and apoptosis induction by Dendosome-encapsulated Curcumin on the Human Gastric Adenocarcinoma AGS cell line**A. Padeganeh<sup>1</sup>, M. Sadeghizadeh<sup>1</sup>, M. N. Sarboluki<sup>2</sup>, S. J. Mowla<sup>1</sup>,<sup>1</sup>Department of Genetics, Faculty of Basic Sciences, Tarbiat Modares University, Tehran, Islamic Republic of Iran, <sup>2</sup>Institute of Biochemistry and Biophysics (IBB), University of Tehran, Tehran, Islamic Republic of Iran.

Gastric Cancer, is a major cause of mortalities with a large number of newly diagnosed cases per annum worldwide. Current modalities for gastric cancer include surgery and chemotherapy, but local recurrence and sever side effects are still unresolved issues in this regard, indicating the necessity of development of safer and more effective therapeutics. Curcumin, is a well known chemopreventive herbal compound. Inhibition of cell proliferation and Apoptosis induction, are proposed mechanisms of action for curcumin.

In the present study, effects of curcumin treatment, along with Dendosome-encapsulated drug were assessed on the survival and proliferation of the human Adenocarcinoma AGS cells. FACS analysis was performed to elucidate the effect of drug treatment on cell cycle and apoptosis status of cells followed by RT-PCR measurement of Oct4 expression profile, as a Cancer Stem Cell marker, assumed to promote cell survival.

Curcumin treatment resulted in a decrease in the G1 population and a significant increase in the number of the apoptotic cells compared to untreated cells.

Further, encapsulation of curcumin into dendrosomes, a novel family of vehicles previously used by our group for transfection and therapy, significantly enhanced the number of apoptotic cells and the G1-population decrease, and resulted in a marked reduction of Oct4 expression.

Our results indicate that Dendosome-encapsulated curcumin, might be a promising chemotherapeutic for inhibition of tumor growth and cell proliferation and that Dendosome encapsulation might enhance the efficacy of treatment while administrating lower amounts of drug.

## P14.10

**The Rat Aldolase B intronic Enhancer augments the Production of Human Coagulation Factor IX minigenes in Cultured Hepatocyte Cell Line**M. Sam<sup>1</sup>, A. Hadad Mashadrizeh<sup>2</sup>, M. Shokrgozar<sup>3</sup>, F. Ataei<sup>1</sup>, A. Amanzadeh<sup>3</sup>, A. Zomorodipour<sup>1</sup>,<sup>1</sup>National Institute of Genetic Engineering and Biotechnology, Tehran, Islamic<sup>2</sup>Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Islamic Republic of Iran,<sup>3</sup>Department of National Cell Bank of Iran, Pasture Institute of Iran, Tehran, Islamic Republic of Iran.

Hepatocyte is main source for production of functional hFIX and suitable host for gene or cell-therapy of hemophilias. For a hepatocyte mediated gene expression, hepatocyte-specific elements such as alpha-1 antitrypsin (AAT) promoter and aldolase B enhancer sequence (ABE) are attractive candidates. To achieve an efficient hepatocyte expression system for production of hFIX, a set of plasmids with various combinations of AAT promoter and ABE enhancer and human beta globin (hBG) introns were used to study the expression of hFIX in HepG2 cells systematically. Comparative analysis of the permanently transfected cells of different minigenes indicates the potentials of the examined elements for specific expression of biologically active hFIX by all the recombinant cells. However various expression levels were obtained, according to the particular constructs used. In the examined conditions the CMV promoter was found stronger than a minimal AAT promoter for the expression of hFIX. Moreover, application of the ABE upstream to both the AAT and CMV promoters augmented the hFIX expression. The enhancer-like effects of the hBG introns on the rhFIX expression level in the cultured media of the transfected cells was also demonstrated. This is a first report that both introns 1 and 2 of hBG in hFIX-cDNA in combination with ABE synergistically contributed to hFIX over-expression by HepG2 cells

The recombinant plasmids and their corresponding cell-lines generated in this work will facilitate studies, dealing with the expression of hFIX in hepatocytes and provided means to investigate the expression of proteins in hepatocytes *in vitro* and *in vivo*.

## P14.11

**A new approach for screening of homologous recombination hot spots into human genome with aim of safe gene therapy**G. Kardar<sup>1,2</sup>, A. Zomorodipour<sup>1</sup>, M. Moin<sup>2</sup>, Z. Pourpak<sup>2</sup>,<sup>1</sup>National Institute of Genetic Engineering and Biotechnology, Tehran, Islamic<sup>2</sup>Immunology, Asthma & Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran.

Gene targeting as a main approach for the treatment of genetic diseases is performed by means of viral and non-viral delivery systems. Both of these systems may lead to complexities, such as oncogenesis, toxicity and possibility of triggering immune responses as well as various genomic rearrangements in the host. Therefore, in order to guarantee the result of a gene targeting experiment with high probability, it is important to identify those potential sites within the human genome, where the integration of a transgene is not harmful to host and the transgene can be expressed efficiently. This approach is done by means of a human genomic library, in which the plasmids carry various overlapping segments of human genomic DNA (3-5 Kb) next to the previously cloned transgene (reporter gene). In order to target various locations of the genome, the linear forms of total recombinant plasmids derived from the amplified genomic library (at least  $4 \times 10^5$  recombinant clones) will be used to transfet a human cell line, where a homologous recombination mediated integration of the plasmids into the genomic DNA is expected. Subsequently, the transfected cells, isolated in selective media, will be screened for the expression of the transgene. Any of cells with successful transgene expression and normal growth will be candidate and subject for mapping of the corresponding integration sites of the transgene. By this new method, we will perform a genome-wide screening and detect several suitable integration sites simultaneously. This may lead to new horizons for safe gene therapy.

**P14.12****Systemic gene therapy for cardiomyopathy and muscular dystrophy of the BIO14.6 hamster**

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The delta-sarcoglycan deficient Syrian hamster strain BIO14.6 is one of the most studied models for inherited dilated cardiomyopathy and muscular dystrophy. This carries a spontaneous deletion of the delta-sarcoglycan gene promoter and first exon. Its lifespan is shortened to 10-15 months because heart slowly dilates towards heart failure. We injected the human delta-sarcoglycan cDNA by AAV2/8 by single intra-peritoneal injection at two weeks of age. We obtained the body-wide restoration of delta-sarcoglycan expression associated with functional reconstitution of the sarcoglycan complex and with significant lowering of centralized nuclei and fibrosis in skeletal muscle. Motor ability and cardiac functions were rescued. Using serotype 2/8 in combination with serotype 2/1, lifespan was extended up to 22 months with sustained heart function improvement.

It is known that corticosteroids have beneficial therapeutic roles in the treatment of Duchenne and Becker muscular dystrophies and sarcoglycanopathies. These drugs allow the maintenance of walking, slowing down the progression of the disease.

At present, all patients with a defined diagnosis of muscular dystrophy are corticosteroid-treated. Our aim is to evaluate the combined effects of gene and glucocorticoid treatments using BIO14.6 hamsters. We treated at the age of 45 days BIO14.6 hamsters using cycles of 0.3 mg/kg deflazacort for 3 weeks followed by 3 weeks of interval without drug. The effects of the interaction were evaluated by serial echocardiography, behavioral tests and histology.

**P14.13****The experience of enzyme replacement therapy for mucopolysaccharidosis VI in Belarus**

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Introduction: Mucopolysaccharidosis VI (MPS VI) is a lysosomal storage disease caused by the deficiency of the arylsulfatase B (ASB). The disease is heterogeneous in clinical presentation and progression. Recently, galsulfase (Naglazyme® [BioMarin]), recombinant human ASB became available as long-term enzyme replacement therapy (ERT).

Goal of this study: to present and compare the safety and efficacy of galsulfase in 2 patients presenting mild and severe phenotypes of MPS VI.

Methods: two female MPS VI patients 29 and 32 years old were treated with 1.0 mg/kg galsulfase for 12 weeks. Urinary excretion of glycosaminoglycans (GAG), endurance, liver and spleen volumes, cardiac and pulmonary function were investigated.

Results: Improved endurance mentioned in 4 weeks of ERT. After 12 weeks patients showed 91 and 18 m gain in 12-minute walk and 55 and 18 stair profit in 3-minute stair climb. Best results were achieved by mildly affected patient. Urinary GAG level decreased by 60% and 90% in patient with mild and severe phenotype respectively in 4 weeks and was sustained thereafter in both. Liver and spleen volumes reduced by 20% in both patients. We found slight improvement in pulmonary and cardiac function in patient with mild disease, but not in severely affected patient. There were no adverse events or allergic reactions within the period of ERT.

Conclusions: ERT was well tolerated in patient with mild and severe clinical presentation of MPS VI. Biochemical response was similar in both patients, while patient with mild disease showed more rapid and significant clinical response.

**P14.14****Phage lambda-derived nanobioparticles; a new generation of eukaryotic gene delivery vehicles**

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Attempts to treatment of diseases caused by genetic deficiencies - gene therapy- have ever faced with various challenges. One of the major challenges in this regard, is selection of a safe and proper carrier. Viral vectors are efficient gene carriers to eukaryotic cells, but, in spite of their high gene delivery efficiency, suffer from frailties such as stimulation of the immune system, likelihood of transformation of the host cells. Bacteriophages have developed significant adaptation to the immune system as they reside every where including the human body. Moreover, it was reported that bacteriophages are safe to eukaryotic cells and possess various capabilities e.g. potential of genetic manipulation and targeting which are the most important features of a potential proper delivery vehicle. In the present study, to evaluate the potency of phages as eukaryotic gene delivery vehicles, the sequence encoding the GFP was inserted into the Lambda ZAP-CMV vector, under the CMV promoter followed by in vitro packaging. The resultant phage particles were further manipulated with rat apo-transferrin as a targeting moiety to formulate directed phage lambda derived nanobioparticles. Further, the transfection efficacy of directed and non-directed phage nanobioparticles into the rat intestinal epithelial cell line IEC-18, was compared using fluorescent microscopy followed by flow-cytometry. Our results highlight the potency of the phage lambda derived nanobioparticles as a new gene delivery route into eukaryotic cells in gene therapy trials.

**P14.15****Generating genetically engineered bone marrow stromal stem cells with reduced rate of cell death after induction of neural differentiation**

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Bone marrow stromal cells (BMSCs) are adult stem cells that have the potential for transdifferentiation into cell types including neuronal cells. Neural-like cells derived from BMSCs carry the potential for repairing degenerative or traumatic CNS injuries. The functional recovery of the CNS promoted by these cells, however, depends on their survival for prolonged periods following grafting into the lesion site. We have investigated the expression profile of the main regulators of neuronal survival /death during neural differentiation of BMSCs. Interestingly, the expression of p75NTR, common receptor of Neurotrophins, is absent in un-differentiated cells but is initiated by 6 hours after induction of differentiation and remained at this level by 12 hours. The expression is completely shot down thereafter. During this period of time (6-12 hrs after differentiation) a substantial proportion of cells undergo cell death via apoptosis. Inhibition of p75NTR receptor using a small interfering RNA revealed a 3.5-fold reduction of apoptosis in neural like cells derived from rat BMSCs. The finding provides a method to increase the survival of the stem cells upon the induction of neural differentiation based on the inhibition of p75NTR receptor and provides the applicable manipulation methods in order to increase the efficiency of cell-based transplantation to cure neurodegenerative disorders.

**P14.16****Surgical aspects in Prader-Willi patients**

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Prader-Willi syndrome (PWS) is the most common genetic cause of life-threatening obesity caused by the lack of a functional paternal copy of 15q11-q13 chromosome. Main clinical features are: hypotonia and feeding difficulties in the neonatal period, hyperphagia and severe childhood obesity at an early age, short stature, varying degrees

of mental deficiency, hypogonadism, scoliosis, osteoporosis and frequent pathological fractures.

The aim of this study is to provide recommendation and clinical pathways for the diagnostic and management of surgical problems of PWS patient.

We encountered a case of a 20 year's old PWS female with typical obesity, short stature and mental retardation. She had a history of congenital hip dislocation, severe muscular hypotonia in infancy and a left femur fracture and delayed healing in childhood. The patient had an inactive lifestyle with low level of physical activity. Clinical exam showed unbalanced thoraco-lumbar scoliosis, lower limb length discrepancy, severe walking and equilibrium maintenance difficulties. Polysomnogram revealed sleep disordered breathing, nocturne sinus tachycardia of 88/ min, apnea-hypopnea index 54.8/ h, mean nocturnal arterial saturation was 96% and total reduction of sleep efficiency to 28%.

Surgical issues are a major concern for PWS patients and regular systematic examination is mandatory. In respect for the good clinical practice procedures we propose an orthopedic treatment, dietary intervention, psychological and educational counseling. We opened a regional data base for patients with PWS for better medical assistance efficient cost management.

#### P14.17

##### The prevalence of prothrombin G20210A mutation by PCR-RFLP in Iranian patients with thromboembolic evidence

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Deep vein thrombosis is one of the leading causes of mortality with an annual incidence of 1 per 1000. Interaction between multiple genetic and environmental risk factor is responsible for thromboembolic tendency. A single nucleotide exchange at position 20210 in the 3' untranslated region of prothrombin gene which results in guanine to adenine transition. This mutation was found as the second most common genetic risk factor for venous thrombosis.

The aim of this study was to determine the prevalence of this mutation in Iranian patients with a history of thromboembolism in national research institute of tuberculosis and lung disease (NRITLD) by PCR RFLP. All thromboemboli patients were from the Iranian population (n=43). The result was compared with matched control group (n=50) that were kidney donor without any known thromboembolic defects. Genomic DNA was used as a template for PCR amplification of exon 14 and PCR product was digested by HindIII enzyme.

The mean age of patient was 52.7±16.1 (range 19-84). They consist of 25 (55.6%) male and 20 (44.4%) female. The mean age of control group was 42 (range 29-60). They consist of 34 male and 16 female. All the patients and controls had homozygote G/G genotype. We had no heterozygous G/A or homozygous mutation (A/A).

In our study none of control subjects and none of patients were carrier for prothrombin A gene mutation which is correlate to finding of two studies in China and Thailand and support the previous report that this mutation is rare in Asia country.

#### P14.18

##### Combined L-Dopa and Selegiline therapy greatly improves the clinical picture in Segawa syndrome: A follow-up study on three siblings with a novel C1475G mutation on Tyrosine Hydroxylase (TH) gene

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Three siblings, of whom the elder are monozygotic twins, born to first cousin parents were referred for neurodevelopmental delay and diffuse dystonia. Initial physical examination of the sibs, twins at age 4<sup>6/12</sup> and the youngest boy 1<sup>9/12</sup> years, revealed body measurements below third centile. They had minor dysmorphic features, such as bifrontal narrowing, downslanting palpebral fissures, low-set ears, up-turned nostrils and retrognathia. Cognitive functions were severely retarded. Increased deep tendon reflexes, diffuse muscle atrophy and spasticity were evident. Absent of eye contact and head control, diffuse dystonia, hypokinesia, choreatetosis and tremor also noted in neurological examination. Molecular testing of tyrosine hydroxylase (TH) gene

revealed a novel mutation, P492R (1475 C>G), which confirmed the diagnosis of Segawa syndrome. After the onset of L-Dopa/Carbidopa (2 mg/kg/day), no response with regard to relieve of symptoms appeared after one month of therapy. Selegiline, an agent selectively inhibits MAO-B, added to the therapy regimen, which in turn, markedly improved the clinical picture. Here, we report the follow-up period of three siblings with Segawa syndrome resulting from a novel mutation in TH gene.

#### P14.19

##### Deferasirox reduced iron-load in patient with Ferroportin disease refractory to phlebotomy: a case report.

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We report a patient with a mutation in the *SLC40A1* gene, which encodes the protein Ferroportin 1 (FPN1) resulting in hemochromatosis type 4 (HFE4), who has been treated with Deferasirox. He was 32 year-old when first seen, asymptomatic but with dusky complexion, and has strong family history suggestive of autosomal dominant hemochromatosis. DNA sequencing from the proband had shown a Cys326Tyr (TGC>TAC) mutation in exon 7 of the *SLC40A1* gene, which is also found in the other affected family members. Although a few family members including himself had previously been treated with regular phlebotomy, his serum ferritin remained unsatisfactorily high (3,624 ng/mL), along with high transferrin saturation (137%; serum iron = 171 µg/dL; TIBC = 125 µg/dL). He was commenced on a therapeutic trial of the oral chelating agent Deferasirox (ICL670). After a period of dosage titration, his serum ferritin declined steadily, as did his transferrin saturation. At the most recent visit, one year after treatment initiation, his serum ferritin decreased to 619 ng/mL, the transferrin saturation to 75% (serum iron = 218 µg/dL; TIBC = 292 µg/dL), and no adverse reaction was noticed. Among the four defined types of primary hemochromatosis, HFE4 is the only type with autosomal dominant inheritance. It also differs from the other HFE's in that most of the iron is deposited in the tissue making phlebotomy ineffective and potentially complicated by anemia. Iron chelating agents therefore seems to be a better option, particularly the orally administered agents as was demonstrated in this patient.

#### P14.20

##### Pharmacogenetic approach to the treatment of SMA patients with valproic acid and carnithin preparations

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Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder, with an incidence in European populations of 1 in 10000 newborns. The telomeric survival motor neuron gene (SMN1) is the SMA disease gene, and the centromeric gene (SMN2) is the main modifier of the disease severity. Recent research suggests that valproic acid (VPA), a commonly used epilepsy drug, may be able to upregulate expression of SMN2 gene and to slow progression of the disease. Since 2003 we were involved in combined clinical trial of VPA + carnithine treatment in SMA patients. Over 60 SMA patients were collected and studied so far with at least half of them being enrolled in the trial. Liver functional activity is monitored and SMN2 gene copy number is determined by means of real-time PCR. Nerve activity testing was performed by means of electroneuromyography. Also the patients were tested on presence of (c.681 G>A) polymorphism in CYP2C19 gene contributing to VPA metabolism and thus to efficacy of treatment. Highly positive curative effects including general health improvements and progress in motion abilities were registered in SMA patients especially of the II type. The results of the study are relevant to elaboration of efficient strategy in SMA patients' treatment.

**P14.21****Higher efficiency of TA-clamp modified single stranded oligonucleotides in targeted nucleotide exchange is not correlated to a lower intracellular degradation****M. Wuepping, D. Kaufmann;***Institute of Human Genetics, Ulm, Germany.*

Specific single stranded oligonucleotides can induce targeted nucleotide sequence correction in an eukaryotic genes *in vitro* and *in vivo*. Our model for investigating the reasons for the low correction rates achieved with this method is the correction of a point mutation in the hypoxanthine-guanine-phosphoribosyl-transferase (*hprt*) gene in the cell line V79-151. Using single stranded phosphorothioate modified oligonucleotides the correction rates of this *hprt* mutation were low but always reproducible. One reason for low exchange rates may be a very fast intracellular degradation of the oligonucleotides. Therefore we compared the exchange rates of different 3'- and 5'-end modified oligonucleotides with their degradation rates. TA-repeat (clamp) modified oligonucleotides showed higher correction rates than those with a GC-clamp and 5'-clamps induced higher correction rates than clamps at the 3'-end. Experiments on the stability of the most effective 5'-TA and 3'-TA-clamp modified oligonucleotide indicated a very rapid cleavage and the occurrence of shortened oligonucleotides in the presence of cytoplasmic and nuclear extracts. The phosphorothioate modified oligonucleotides were more stable, but their correction rates were lower. We suggest that there is no direct correlation between the biological stability of the full length oligonucleotides and the exchange rates achieved.

**P14.22****Rapamycin treatment of a girl with double phakomatosis: tuberous sclerosis and neurofibromatosis type 1****K. Mayer<sup>1</sup>, H. Seidel<sup>2,3</sup>, A. Wiemer-Kruef<sup>4</sup>, I. Rost<sup>1</sup>, M. Staehler<sup>5</sup>, M. Fischereder<sup>6</sup>;**<sup>1</sup>*Center for Human Genetics and Laboratory Medicine, Martinsried, Germany,*<sup>2</sup>*Institute of Human Genetics, Technical University, Munich, Germany, <sup>3</sup>Institute of Human Genetics, Ludwig Maximilians University, Munich, Germany,*<sup>4</sup>*Epilepsy Centre Kork, Clinic for Children and Adolescents, Kehl-Kork, Germany,*<sup>5</sup>*Department of Urology, Ludwig Maximilians University, University Hospital Grosshadern, Munich, Germany, <sup>6</sup>Department of Nephrology, Medical Polyclinic, Ludwig Maximilians University, Munich, Germany.*

Tuberous sclerosis (TSC) and neurofibromatosis type 1 (NF1) represent the two most common neurocutaneous disorders. Both are autosomally dominantly inherited with well-delineated genetic and clinical findings. However, the simultaneous presentation in one patient is quite rare. We report a 13 year old girl with the clinical diagnosis of TSC including skin, brain and kidney lesions and additionally an increasing number of cafe-au-lait spots. Molecular analysis revealed a *de novo* TSC2 mutation and a NF1 mutation inherited from the mother.

The gene products of TSC1 and TSC2, hamartin and tuberin, respectively, form a complex and inhibit the mammalian target of rapamycin (mTOR) in the correspondent signalling pathway. The drug rapamycin has been shown to suppress mTOR signalling which is activated in renal angiomyolipoma due to TSC mutations. The patient has been treated with rapamycin for six months in order to reduce angiomyolipoma volume prior to organ preserving renal surgery. The achieved significant reduction of tumour volume allowed partial nephrectomy and complete AML resection. As an additional benefit, cognitive functions improved as a consequence of decreased epileptic activity.

NF1 is caused by loss-of-function mutations of the NF1 gene encoding neurofibromin, a RasGAP. In NF1 deficient human tumours, Ras and mTOR are activated through cross-talk of Ras/MAPK and PI3K/Akt/mTOR signalling pathways. To our knowledge this is the first patient with TSC and NF1 treated with rapamycin. Although she currently presents only cafe-au-lait spots potential future rapamycin treatment focussing on NF1 lesions will show if the drug also exhibits a viable therapy for NF1.

**P14.23****Particularity of the therapy in a case with Turner syndrome associated with juvenile rheumatoid polyarthritis****M. Cevei<sup>1</sup>, D. Stoicanescu<sup>2</sup>, D. Farcas<sup>1</sup>;**<sup>1</sup>*Faculty of Medicine and Pharmacy, Oradea, Romania, <sup>2</sup>University of Medicine and Pharmacy, Timisoara, Romania.*

Juvenile rheumatoid arthritis is the most common type of childhood arthritis. It is an autoimmune, chronic disease that most commonly causes inflammation and tissue damage in joints and tendons. The most common features are: joint inflammation, joint contracture, joint damage and/or alteration or change in growth. Other symptoms include joint stiffness following rest or decreased activity level and weakness in muscles and other soft tissues around involved joints. Turner syndrome is a chromosomal disorder with characteristic physical abnormalities, such as short stature, signs of ovarian failure and also skeletal dysplasia. We present the case of an 18 years old girl diagnosed with juvenile rheumatoid arthritis at the age of 3 years. During childhood she was also diagnosed with Turner syndrome. Besides replacement therapy, she was initially treated with nonsteroidal anti-inflammatory drugs and prednisone and then with methotrexate and Enbrel. Treatment focused on preserving physical activity to maintain full joint movement and strength, preventing damage and controlling pain. Medical rehabilitation treatment was associated to the biological therapy, the main objective being maintaining mobility and functional parameters for an active life. As there is an association of these two conditions, the standard therapy could not be applied, she could not receive the efficient doses of drugs, and the rehabilitation program has been modulated according to the existing statural deficit and coexistence of modified joints with limited range of motion.

**P15. Laboratory and quality management****P15.01****The POCEMON (Point-Of-Care MONitoring and Diagnostics for Autoimmune Diseases) project: building a Lab-On-Chip centered on Rheumatoid Arthritis and Multiple Sclerosis.****S. Lupoli<sup>1,2</sup>, C. Cosentino<sup>2</sup>, V. Tieran<sup>2</sup>, F. Taddeo<sup>2</sup>, S. Atkinson<sup>3</sup>, A. Barton<sup>4</sup>, A. Wilson<sup>3</sup>, D. Plant<sup>4</sup>, J. Maxwell<sup>5</sup>, I. Chumakov<sup>6</sup>, F. Kalatzis<sup>6</sup>, K. Schicho<sup>7,8</sup>, H. Gruessinger<sup>8</sup>, F. Macciardi<sup>1,2</sup>;**<sup>1</sup>*INSPE, HsR Scientific Institute, Milan, Italy, <sup>2</sup>University of Milan, Milan, Italy,*<sup>3</sup>*University of Sheffield, Sheffield, United Kingdom, <sup>4</sup>arc Epidemiology Unit, University of Manchester, Manchester, United Kingdom, <sup>5</sup>Pharnext S.A., Paris, France, <sup>6</sup>Unit of Medical Technology and Intelligent Information Systems, University of Ioannina, Ioannina, Greece, <sup>7</sup>Medical University of Vienna, Cranio-Maxillofacial and Oral Surgery, Vienna, Austria, <sup>8</sup>PCS Professional Clinical Software GmbH, Klagenfurt, Austria.*

POCEMON is a large-scale integrated project founded from the European Commission (FP7-ICT-2007-216088). The aims of the project are to develop an integrated diagnostic platform mainly dedicated to Rheumatoid Arthritis (RA) and Multiple Sclerosis (MS) in a first stage by combining Lab-On-Chip technology, DNA microarray genotyping, microelectronics, mobile devices, intelligent algorithms and wireless communications.

MS and RA are two progressive autoimmune diseases and are causes of disability in young adults. Considering the social relevance of MS and RA, it is extremely important to improve the timing and confidence of the diagnosis. According to this vision the POCEMON project can be a real milestone for the improvement of life style of many patients.

In a first (discovery) phase, we are performing a case/control whole genome association study, mostly centered to identify HLA (Human Leukocyte Antigens) and other potentially relevant susceptibility genes for the two diseases. For the discovery phase of RA, we are using a homogeneous North-European population (with 800 cases and 800 controls). The discovery is then followed by a confirmatory phase, where the "best" SNPs are evaluated in a second independent sample (2000 cases affected by RA or MS respectively and relative controls), from three separate cohorts.

The genotyping phase for RA, using Illumina HumanCNV-370, is concluded. Plink 1.05 is used for QC of genotyping data, single marker association analysis and permutations. The analysis is ongoing. Initial findings pointed to several susceptibility genes across the genome involved in the disorder other than confirming a role of HLA.

**P15.02****Whole genome and transcriptome amplification in large biobanks**N. Klopp<sup>1</sup>, T. Illig<sup>1</sup>, C. Korfhage<sup>2</sup>, H. Wichmann<sup>1</sup>;<sup>1</sup>Helmholtz Zentrum München, Munich-Neuherberg, Germany, <sup>2</sup>QIAGEN GmbH, Hilden, Germany.

Biobanks are a key resource in unravelling the molecular basis of diseases, identification of new targets for therapy and improvement of attribution in drug discovery and development. The scientific trend in biobanking shows the need for stable techniques for amplification of biomaterials, which can be used for samples stored under very different conditions. The focus of the project is the standardisation and validation of the innovative techniques of whole genome amplification (WGA) and whole transcriptome amplification (WTA) in the context of biobanks. A general standardized protocol for WGA and WTA procedures that use Phi29-DNA-polymerase in biobanking will be developed. The major aims of our project are:

1. To establish standardized WGA protocols for large biobanks
2. To develop standardized WGA tools to recover genomic DNA, which is in plasma or serum samples and from FFPE- tissue or blood spots
3. To optimize the WGA procedure by extensive quality control measures of WGA products
4. To develop and establish WTA of large biobank samples
5. To optimize WTA procedures by extensive quality control of WTA products

Furthermore, the concept of the project is to transfer the results of WGA and WTA solution to national and international organisations in the field of biobanking. The development of the proposed, innovative and specialized tools and customized solutions will help to expand and secure biobanks.

**P15.03****Finding the right song : - Introducing clinical test criteria for Cystic Fibrosis (CFTR) and Fragile X (FMR1) molecular testing.**P. W. Lunt<sup>1</sup>, & Project Team<sup>2</sup>;<sup>1</sup>(Bristol) Clinical adviser to UK Genetic Testing Network (UKGTN), Bexley (ukgtn@bexley.nhs.uk), United Kingdom, <sup>2</sup>UKGTN, Bexley (ukgtn@bexley.nhs.uk), United Kingdom.

Due to the volume of requests, despite low individual cost, tests for Cystic Fibrosis (CFTR gene) and Fragile X (FMR1 gene) make up 2 of the leading 5 conditions contributing to total UK cost of DNA testing in clinical service. The UKGTN promotes the development and use of clinical testing criteria for single gene disorders to ensure clinical appropriateness of newly available DNA tests offered to the National Health Service. Following a strategic decision to develop testing criteria for the commoner high volume conditions, two workshops of experts were organized in 2008 to formulate clinical testing criteria for CF (or CFTR-related disorders) and FraX (and related disorders). For CF, 7 different clinical scenarios (fetal echogenic bowel; symptomatic child; adult with bronchiectasis; pancreatitis; absent vas; carrier testing; reproductive donor) and for FraX, 5 scenarios (learning-disabled male child; female; adult; FXTAS; POF) were separately considered, and criteria developed. Most dovetail to relatively tight clinical definitions of the target population (eg. bronchiectasis must be CT scan proven with a not-normal sweat test and/or appropriate bacterial flora; fetal echogenic bowel must be 'as bright as bone' as an isolated anomaly and with obvious other causes excluded; ovarian failure must be at <40yrs). However, it is clear that whereas the role of CF testing is to make a diagnosis, and criteria could rationalise requests, FraX testing is largely used as a first line exclusion test where rationalisation is less applicable. Implementation of the criteria will, however, present a separate challenge.

**P15.04****UK genetic testing network and the evolution of the gene dossier process**

S. A. Stenhouse, on behalf of the UKGTN gene dossier working group; West of Scotland Regional Genetics Service, Glasgow, United Kingdom.

The UK Genetic Testing Network was established in 2002 to ensure reliable, high quality, equitable services for patients and their families who require genetic advice, diagnosis and management. The Gene

Dossier<sup>(1)</sup> was developed to provide a formal process for the evaluation of new genetic tests and was first piloted in 2003. The Gene Dossier Working Group was set up in 2004 to provide a dedicated forum for the evaluation of dossiers and is comprised of clinicians, patient representatives, scientists and members of the UKGTN Project Team.

The gene dossier incorporates the key concept that 'a genetic test' is a test that detects

- A particular genetic variant (or set of variants)
- For a particular disease
- In a particular population
- For a particular purpose

From these principles it follows that the test is evaluated as a component of a patient care pathway rather than in isolation.

Genetics is a rapidly advancing field and the gene dossier process needs to be adaptable to the changing needs of patients and the network laboratories. This has led to an evolution of both the dossier and the evaluation process which will be described.

(1) Kroese, M, et al. (2007) EJHG, 15, 917-921

**P15.05****Hardware and Consumable Innovations for a New Genetic Analysis System**S. R. Berosik<sup>1</sup>, A. Chhibber<sup>1</sup>, C. J. Davidson<sup>1</sup>, R. N. Fish<sup>1</sup>, J. R. Goudberg<sup>1</sup>, S. C. Hung<sup>1</sup>, B. F. Johnson<sup>1</sup>, E. S. Nordman<sup>1</sup>, R. A. Padilla<sup>1</sup>, A. A. Pradhan<sup>1</sup>, A. C. Felton<sup>1</sup>, R. R. Santhanam<sup>1</sup>, L. K. Joe<sup>1</sup>, M. Yamazaki<sup>2</sup>;<sup>1</sup>Life Technologies Corporation, Foster City, CA, United States, <sup>2</sup>Hitiachi High Technologies, Naka, Ibaraki, Japan.

The genetic analysis platform by which all sequencing technology is compared for accuracy and quality of data generation continues to be the capillary electrophoresis (CE) platform. A genetic analysis system that builds upon the industry standard of CE has been developed that incorporates hardware and ease-of-use improvements. This newly designed system will support DNA sequencing and fragment sizing applications, and provides scientists with medium-throughput technology for use in research validated environments.

The steps from system set-up to base-called or size-called data results have been facilitated with hardware functionality and ease-of-use enhancements designed into this new CE system. We will discuss a host of advancements on this new CE system including: an improved polymer delivery pump design, ready-to-use consumables and containers, 6-dye detection functionality, radio frequency identification- (RFID) consumable tracking integration, plate base flexibility, increased throughput, improved power efficiency, peak height normalization, intuitive user software, and integrated primary analysis software. Run modules have been developed for analysis of small amplicon resequencing to long-read sequencing, as well as fragment sizing modules for numerous applications.

**P15.06****Performance of the KB™ Basecaller for a New Sequencing System**

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Life Technologies Corporation, Foster City, CA, United States.

A sequencing system that builds upon the industry standard of capillary electrophoresis has been developed to help streamline the generation of high quality pure- and mixed-base sequencing content, while also providing scientists with long-, medium-, or short-read sequencing options. The thrust of this newly designed system provides scientists with low-to-medium throughput DNA analysis requirements with a capillary electrophoresis platform generating high-quality data for use in both research and regulated laboratory environments.

Development of a new capillary electrophoresis DNA analysis platform includes the development and validation of the basecalling capability for these systems. Dye-labeled DNA fragment mobility correction as well as the capability of generating high-quality, highly-accurate sequencing content is essential for successful sequencing projects.

Below we highlight a process used to evaluate the performance of this new system, including validation of the KB™ Basecaller basecalling algorithm. The process starts with the creation of the mobility correction file content and includes the data generation and validation of the pure- and mixed-basecalling abilities of the KB™ Basecaller for the

system using more than 7,800,000\* independently called bases with quality value (QV) 20 or better data (\*data estimate excludes the calls < QV20 which are also used).

#### P15.07

#### System Validation of a New Real Time PCR and High Resolution Melting instrument: the LightScanner32 (LS32)

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*Idaho Technology, Inc., Salt Lake City, UT, United States.*

Rapid air-thermocycling was introduced by Idaho Technology in 1991, resulting in the development of the LightCycler in 1996. Licensed to Roche (1998), the LightCycler is now commonplace throughout the world with an install base >7000. High-resolution melting was introduced by Idaho Technology in 2003 with the HR-1 instrument and is now a validated technology for mutation scanning and genotyping. The LightScanner32 (LS32), capable of both rapid real-time PCR and high-resolution melting, has been developed by Idaho Technology and launched in February 2009. LS32 combines features of the LightCycler and HR-1 instruments (see Table 1). System validation tested instrument and software functionality independently and as integrated components. Instrument control and analysis modules were tested with independent 16-rotor experiments as part of the formal system validation plan. Integrated system testing included: multiplex qPCR with color-compensation; qPCR dynamic range, precision, and accuracy; high-resolution melting; multi-users and database's; customizable data reports. Over 100 experiments representing specific test cases were performed for each design input and product specification. All design inputs and product specifications related to system performance and experimental applications were successfully verified with the planned test cases. System validation resulted in final modifications to software and the operator manual, with any potential software modifications validated by re-running the appropriate test cases. LS32 system performance was documented via Idaho Technology's quality system and approved for launch in February 2009.

Instrument Comparison			
	LightCycler(Roche)	HR-1(IdahoTech)	LS32(IdahoTech)
Amplification	Yes	No	Yes
Analysis Mode	qPCR, Melting Curves	Hi-Res Melting	qPCR, Melting Curves, Hi-Res Melting
Acquisition Mode	Single/Step/Continuous	Continuous	Single/Step/Continuous
Data Resolution	4.1 pts/C°	400 pts/C°	400 pts/C°
Sample Capacity	32	1	32

#### P15.08

#### The MLPA-dHPLC procedure to analyse the NF1 gene

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The identification of mutations in the NF1 gene causing type 1 neurofibromatosis (OMIM-162200) is still presenting a considerable amount of work mainly because of the large size of the gene and the restricted number of recurrent mutations. The high frequency of NF1 which affect 1 in 3500 individuals lead us to choose two complementary methods for NF1 gene analysis:

- the multiplex ligation-dependant probe amplification (MLPA) for the large deletion and duplication detection (P081 and P082 - P122C1)
- and the automated denaturing high performance liquid (dHPLC) screening method.

The MLPA method was validated by the detection of 19 known large NF1 gene deletions. We also tested 39 different mutations that would interfere with the MLPA results.

The dHPLC was optimised for a rapid screening of the 60 exons and the splice junctions of the NF1 gene. The dHPLC conditions were validated by the detection of 260 known variants located in two thirds of the NF1 exons. We also evaluated the sensitivity of dHPLC for somatic mosaicism mutation.

The sensitivity was evaluated in a MLPA/dHPLC analysis of a panel of 150 unrelated french NF1 patients with at least two consensus diagnostic criterias.

Seven large deletions were first detected by P081/082/P122C1 MLPA (6 total and 1 partial).

Mutations were identified in 136 among the 143 remaining patients with a global mutation detection rate of 96% [CI95%: 91-98].

Our results confirm that the association of the MLPA and dHPLC techniques provides an accurate and fast method for the identification of NF1 mutations.

#### P15.09

#### Information on quality assurance in genetic testing in Europe: Orphanet/ EuroGentest

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Given that the outcome of genetic testing has a great impact on the life of patients and their entourage, the quality of genetic testing is of utmost importance. Since December 2008, the EuroGentest portal of the Quality Assurance database moved to the Orphanet website. Data about the quality management of laboratories offering medical genetic testing are now linked to their tests and contact details. To ensure the highest possible reliability of the quality assurance data, replies of laboratories are validated by comparison with EQA providers and accreditation bodies by EuroGentest, prior to dissemination via Orphanet database. Currently, the database includes data from 1,308 laboratories. Quality data comprise laboratory accreditation status with a link to the accreditation scope, participation in genetic external quality assessment and the presence of a quality manager.

With the developing awareness of the central role of QAU, we believe that the uptake of quality data in Orphanet will benefit *laboratories* by encouraging and providing recognition of their investment in QAU and by the possibility of a better informed choice for referral of tests, *consumers* (patients, doctors, laboratories, etc.) by a greater transparency and a possibility of a better-informed choice, and *quality organizations* (EQA providers, accreditation bodies etc.) by greater visibility and recognition of their roles.

Orphanet is continuously updated and participation in the database is freely open to all European laboratories offering human medical genetic testing ([www.orpha.net](http://www.orpha.net)). If you have any further questions concerning quality data, please contact the EuroGentest team at QAUSurvey@eurogentest.org.

#### P15.10

#### The role of EuroGentest and CF Network in measuring the improvement of quality assurance in genetic testing laboratories

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Laboratories across Europe are increasingly evolving towards implementation of quality assurance (QAU) and ISO 15189:2007 is being widely implemented by genetic testing services. The EuroGentest workshops on QAU and the CF Network cystic fibrosis (CF) external quality assessment (EQA) scheme provide useful instruments to examine the evolution of quality in laboratories.

35 institutions that participated in the workshops were surveyed about their accreditation status and implementation of different "quality parameters". Accreditation changed from 37% of the laboratories in 2005 to 49% in 2007. The majority of the non-accredited laboratories initially participated in EQA schemes (72% in 2005, 83% in 2007), whereas technical aspects such as validation of methods (0% in 2005, 22% in 2007) tend to be addressed later in the move towards accreditation. Overall, implementation of all the parameters surveyed increased with time. A follow-up survey will be performed in 2009.

152 laboratories participated in the CF EQA scheme in each of 2005, 2006 and 2007. Improved error rates in genotyping (4% in 2005, 2% in 2007) and interpretation errors (14% in 2005, 7% in 2007) are encouraging. Similar improvements are apparent for the inclusion in reports of elements required by ISO 15189:2007, such as patient name (98% in 2005 and 2007), sample number (86% in 2005, 89% in 2007) and gender (53% in 2005, 63% in 2007). 2008 scheme data will be analysed.

These results reveal a tendency for quality improvement in laboratories participating in continuous education and EQA, which should lead to improved services and patient care.

#### P15.11

#### Quality assurance and management in clinical cytogenetics laboratories: The role of a technical assessor

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Obtaining accreditation from reputed organization has become a concern of many clinical cytogenetics laboratories across the world for assuring a standard operating system. For clinical laboratories ISO 15189 standard is generally followed for accreditation. The technical assessors in the field recruited by the accreditation organization assess the quality system and documents of laboratory-operation with the help of the check-list which is specifically designed by the accreditation organization for the purpose. It has been experienced that the experts engaged in routine or molecular diagnostic service with some exposure in the field of clinical cytogenetics are employed for assessment. However, such assessors fail to detect the diagnostic errors and make specific comments on the inaccurate ongoing system and its further improvement. It is true that conventional cytogenetics depends on skilled expertise and dedicated involvement for extraction of correct information on the constitutional or acquired anomalies and making diagnostic interpretation on the present status and its future implication. Involvement of inexperienced persons at technical and/or supervisory level in lab operation and assessment as well will misinterpret the standard of service and accreditation. The technical assessor shall be a qualified expert with enough experience in clinical cytogenetics. Failing which the accreditation might help the audited laboratory to increase the work-load/business; however, the quality of diagnosis will be compromised in such accredited laboratories. It is true that the constitutive errors cannot be corrected, but accurate result can prevent the recurrence of anomalies, while acquired anomalies can be treated in many cases of hematological malignancies.

#### P15.12

#### EuroGentest: A collaborative network aimed at improving the quality of genetic testing

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EuroGentest, a European Commission funded Network of Excellence, aims to improve and harmonize the quality of genetic testing (GT) across Europe. EuroGentest includes a Quality Management (QM) Unit whose main objective is to ensure that all genetic testing services are offered within a structure that will assist Governments, regulators and professional bodies to fulfil their responsibility to the public. The Quality Management Unit has:- (1) organized 13 quality workshops for genetic laboratories, covering 5 topics, where participants learn about the requirements of a QM system and share experience of problem-solving; (2) set up a database listing tests, accreditation status and EQA participation of laboratories, enabling users to find accredited laboratories and specific genetic tests ([www.orpha.net](http://www.orpha.net)); (3) increased the range of EQA schemes available for CEQA, EMQN & ERNDIM, organized meetings of EQA scheme organizers - consequently some harmonisation and merging of EQA schemes has occurred; (4) through workshops developed and produced disease-specific best practice guidelines; (5) produced draft guidelines for the validation of assays for clinical use and DNA extraction and MLPA protocols have been validated and (6) provided guidance on the proper use of reference materials (RMs) with a priority list drawn up and RM producers have been assisted with sourcing patient materials, design of RM panels, validation of materials and the adoption of the Fragile

X RMs by WHO.

EuroGentest's efforts have led to an increased awareness of the importance of quality assurance in GT, and the projects are helping to improve the quality of GT across Europe.

#### P15.13

#### Genetic testing in Europe: major differences from one country to another

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Genetic tests are now offered internationally, through both public and private sector genetic testing services. Physicians prescribing these tests and biologists receiving the samples need to know which tests are available, where they are performed and whether the identified laboratories meet quality standards. To fulfil this need, [www.orpha.net](http://www.orpha.net) was launched ten years ago to set up a database of clinical laboratories in the field of rare diseases. The data collection covered 1 country in 1997, 15 in 2003, 26 in 2006 and 38 in 2009. This major effort was enabled thanks to resources from the EC DG for public health. In collaboration with the EuroGentest NoE, information on quality management has been added over the past three years. To obtain information on genetic testing in Orphanet, it is possible to search by disease name or by gene (symbol or name in English) in addition to the traditional search by name of laboratory or professional. The information provided on laboratories includes data on quality management. Currently, 1308 laboratories offering tests for 2991 diseases are registered on the Orphanet database. The test offer differs greatly from one large country to another: Germany (1940 diseases), France (1630 diseases), Italy (1110 diseases), UK (845 diseases) and Spain (740 diseases). Medium and small-sized countries have a test offer ranging from 3 to 956 diseases. This situation explains the large cross-border flow of specimens and underlines the need to organise services at the European level.

#### P15.14

#### Evaluation of four automated mutation detection programs for clinical re-sequencing.

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Clinical re-sequencing of large genes by capillary electrophoresis requires software capable of accurate and rapid variant detection. We identified four commercially available programs, Variant Reporter 1.0 and Seqscape 2.6 (Applied Biosystems), Mutation Surveyor 3.24 (Softgenetics) and SeqPilot 3.2.1.2 (JSI Medical Systems) and evaluated their ability to: detect and flag various variants, process large datasets, utilize HGVS nomenclature, identify regions of interest (ROI) and de-convolve two alleles. Parameters such as software ease of use, network performance, audit trail capability and operational costs were also considered. After preliminary testing, two programs (Seqscape and SeqPilot) met our minimum diagnostic requirements and were subjected to a variant analysis of over 2,000,000 bp of bi-directional sequence (ie. over 4,000,000 bp in total). Both programs had a bi-directional false-negative rate of 0%, but also have the potential to fail to alert the user to variants located in a single direction (ie. variants under primer sequences or miscalls due to incorrect assignment of base spacing). Additional features important to a diagnostic laboratory such as electropherogram peak statistics, a mutation database, audit trail capacity, a client-server application, and the ability to de-convolve heterozygous indel mutations into two alleles are provided by the SeqPilot software. Therefore, we have concluded that of the four programs evaluated, the SeqPilot software provides the greatest overall utility for clinical re-sequencing of large genes.

## P16. Molecular and biochemical basis of disease

### P16.01

#### Molecular diagnosis for Alström Syndrome

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**Introduction:** Alström Syndrome (AS) is a monogenic recessive disorder featuring an array of clinical manifestations: systemic fibrosis and multiple organ involvement, retinal degeneration, hearing loss, childhood obesity, diabetes mellitus, dilated cardiomyopathy, and pulmonary, hepatic, and renal failure. Currently, 300 individuals with AS have been identified in 44 different countries. AS is caused by mutations in the ALMS1 gene, which comprises >224 kb of genomic DNA, 23 exons and encoding a predicted 461.2 kDa protein with unknown function. CGC Genetics is the reference laboratory for Alström Syndrome International. Here we report our experience and contribution for the identification of new Alström patients.

**Method:** In 2004-2008, we received 52 samples for ALMS1 gene analysis from 8 different countries, from patients with clinically established diagnosis and also from patients clinically suspected for AS. Our approach for the molecular genetic testing was the complete sequence analysis of exons 10 and 16, plus a portion of exon 8.

**Results:** Causative mutations (missense) were identified in 13 patients in both alleles and also 6 new nonsense mutations. In 6 patients we found a causative mutation only on one chromosome.

**Conclusion:** Being a rare disorder and not commonly known, AS is most probably underdiagnosed, has delayed diagnosis or is even misdiagnosis. Genetic test allows an earlier diagnosis of the disease. Efforts are being made to increase the detection rate by detecting large deletions.

### P16.02

#### Analysis of the candidate gene PDE6B in families with Bardet-Biedl syndrome

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Bardet-Biedl syndrome (BBS, MIM 209900) is a rare multiorganic disorder which patients manifest a variable phenotype that includes retinal dystrophy, polydactyly, mental delay, obesity and also reproductive tract and renal abnormalities. Until now 12 genes (BBS1-BBS12) have been involved in 70% of the families, indicating that additional mutations in known BBS genes and new BBS genes remain to be identified. Previous studies\* have pointed out, by different methods (homocigosity mapping, comparative genomics and gene expression analysis), a total of 19 potential candidate BBS genes. One of them was PDE6B (Rod cGMP-specific 3',5'-cyclic phosphodiesterase beta-subunit), this gene (4p16.3) codifies for the primary effector enzyme in the photo-transduction cascade in the rods, and it is implicated in congenital stationary night blindness and recessive retinosis pigmentaria.

We analyzed the coding sequence of the PDE6B gene in 16 BBS families. In the probandus of the families, we found 7 sequence variants, one in homozygous state (p.V320I, c.958A>G), and six in heterozygous state, including a novel missense variant (p.G352V, c.1055T>G), a nonsense variant (p.T305T, c.915G>A) and several intron sequence variants with no consequences in the splicing process (IVS9-117 C>T, IVS18+63 G>A, IVS11+21C>T and IVS22+11A>G)

The familial segregation and the nature of the variants showed that they are no disease causing mutations. So, we concluded that PDE6B is not, at least in the studied BBS families, a gene implicated in the Bardet-Biedl syndrome.

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\*Nishimura DY et al. 2005. Am J Hum Genet. 7(6):1021-33.

### P16.03

#### Identification of 30 novel mutations in the Bardet-Biedl syndrome (BBS) genes: the burden of private mutations in extensively heterogeneous diseases

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France.

Bardet-Biedl syndrome (BBS) is an autosomal recessive ciliopathy defined by progressive retinal degeneration, obesity, cognitive impairment, polydactyly and kidney anomalies. BBS is genetically heterogeneous with 14 genes identified to date, which account for ~75% of affected families. BBS1 and BBS10 each account for ~20% of the mutational load, BBS12 for about 8% whereas each of the other genes accounts for ≤5% of the cases (or even for BBS11, 13 and 14 each in a single family).

The genetic heterogeneity is a burden for identifying mutations as the full sequencing of the BBS coding sequences implies >150 amplicons and is time-consuming with routine techniques of diagnostic laboratories.

We analyzed a cohort of 174 families, using various strategies, including SNP array for initial homozygosity mapping of candidate loci. The latter can in some cases detect consanguinity unknown to the family. Mutations have been identified in 135 families (78%) whereas 39 families (22%) have no mutation detected and are explored for undetected mutations (deletions, promoter) and new gene identification. We have recorded 83 mutations in 11 BBS genes, of which 30 mutations are novel, confirming the high level of private mutations in this very heterogeneous condition and highlighting the difficult task of routine mutation identification in the perspective of genetic counseling for the families. We pinpoint the absence of BBS11 mutations, leaving this gene linked to BBS through a single missense mutation reported in one family, while several other mutations are responsible for the very different phenotype of limb girdle muscular dystrophy (LGMD2H).

### P16.04

#### A homozygous mutation in BBS2 is responsible for Bardet-Biedl syndrome in the Hutterite population

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Bardet-Biedl syndrome (BBS) is a genetically heterogeneous rare autosomal recessive ciliopathy characterized by retinopathy, obesity, genitourinary malformations, polydactyly and cognitive impairment. At least 14 BBS genes have been identified to date. A Hutterite boy was identified in infancy with polydactyly and renal cysts. Now age 15 years, he has typical features of BBS: learning disabilities, night blindness with a rod-cone dystrophy and obesity. The Hutterites are a genetically isolated population of 40,000 individuals derived from 89 common founders. Thus, we assessed the known BBS loci for evidence of identity-by-descent from a common ancestor. Genotypic information from his three unaffected sibs was used to rule out regions of homozygosity in the patient present due to chance. Initially, microsatellite markers flanking the known BBS genes were used and segregation analysis made them unlikely candidates. Genome-wide analysis using a 10K SNP microarray identified two regions of homozygosity present in the patient but absent in his sibs. One of the regions included the BBS2 gene on 16q21, initially ruled out using microsatellite analysis. Sequence analysis identified the splice variant c.472-2A>G; RNA analysis confirmed aberrant splicing. These findings demonstrate a pitfall in using microsatellite markers in homozygosity mapping: homozygosity at a locus can not be excluded in the presence of heterozygosity at a closely flanking microsatellite marker. The misleading marker in this case was approximately 207 kb from the BBS2 gene.

### P16.05

#### The natural history of primary arrhythmia syndromes

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**Introduction:** Whereas for most hereditary arrhythmia syndromes the natural history is unknown, physicians face an increasing need for such data, when decisions on treatment options have to be taken for the rapidly increasing number of asymptomatic gene carriers in various disorders with a definite but ill defined risk of sudden cardiac death.

**Methods:** We used the Family Tree Mortality Ratio (FTMR) method to study all cause mortality in times when the disease was not elucidated and patients were untreated (i.e. the natural course). Four large pedigrees dating back to the 19<sup>th</sup> century were obtained: a pedigree with carriers for LQTS1 (n=55 persons), LQTS2 (n=76), LQTS3 (n=179) and CPVT (n=178). All persons in the pedigrees had a 100 % or 50% probability of carriership. All cause mortality was compared to the general Dutch population in similar time intervals (SMR).

**Results:** For LQTS1; there was significant excess mortality in males (SMR 1.9, CI 1.2-2.9), especially in the age categories 1-9 years (SMR 3.4, CI 1.3-7.4). For LQTS2 patients there was significant excess mortality between 5-14 years (SMR 4.8, CI 1.3-12.3). For LQTS3; severe excess mortality occurred in the age categories 10-49 (SMR 3.8, CI 2.4-5.7). For CPVT patients there was significant excess mortality in the age category 20-29 years (SMR 3.91, CI 1.27-9.12).

**Conclusions:** We demonstrate by using the FTMR method significant excess mortality in specific age categories that differ between diseases. This information might help to guide timely screening and treatment of (asymptomatic) patients who are carrier of an inherited (cardiac) disorder.

#### P16.06

##### Obstructive Left-Sided Cardiac Lesion in A Saudi Family

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Congenital cardiac defects are relatively common and occur in about 1 in 200 births (Harper, 2004). They are known to be associated with many well described genetic disorders. Here, we reviewed a highly consanguineous Saudi family with left-sided obstructive cardiac lesion. It was characterized by a small transverse aortic isthmus and small aortic valve annulus that was affecting several members. The segregation of this observed congenital anomalies was suggestive of an autosomal recessive mode of inheritance. Karyotype and FISH study for chromosome 22q11.2 was normal. There was no documented associated malformation.

Carrying out a genetic study to identify the disease causing mutation is necessary in order to provide appropriate genetic counseling interventions.

#### P16.07

##### Progress in development of UK inherited cardiovascular conditions (ICC) services in the light of recent scientific advances

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There are many different forms of rare inherited disease that cause sudden cardiac death, arrhythmias and other cardiovascular conditions. Together, individuals with these diseases represent a significant component of both cardiac and genetics services. In the last ten years rapid advances in our understanding of the pathological basis of inherited cardiac disease, clinical features and associated risks has created the potential for the effective clinical management of patients and their families. In particular, highly specialised services combining both cardiology and genetics expertise are required. In 2008 -09 the PHG Foundation led a group of key experts and stakeholders including geneticists, cardiologists, health service commissioners and managers and representatives of relevant voluntary organisations, in a review of UK provision in inherited cardiovascular conditions. Findings will be presented including some estimates of current service need and shortfall in the UK, a review of current services and agreed important features of a high quality specialist ICC service. The potential impact of new technologies will also be considered.

The presentation will include key recommendations for development of ICC services in the UK that will be applicable in other European countries.

#### P16.08

##### Molecular testing of Cardiomyopathy Families in the West of Scotland

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An Inherited Cardiac Clinic was established in the West of Scotland in 2007. Genotyping has been carried out in 121 families, 84 with hypertrophic cardiomyopathy (HCM), 33 with definite familial dilated cardiomyopathy (FDCM) and 4 with left ventricular non-compaction (LVNC). In HCM families we have detected sarcomeric protein mutations in 59 (70%) , 58% of these are in MYBPC3, 27% in MYH7 and 7% in TNNT2, 3 families have two mutations in MYH7/MYBPC3. In sporadic HCM the detection rate was only 40%, in those with a FH it was 84%, and in those with FH of sudden cardiac death detection rate 93%.

In 33 with FDCM, 30 families have had sarcomeric proteins analysis, seven in addition had lamin A and three families had Lamin A only. Only 6 mutations were detected (18%), 2 MYH7, 1 TNNT2, 1 TNNT3 and 2 Lamin A mutations (+ one variant of unknown significance).

In 4 families with LVNC we have detected 2 sarcomeric mutations (1 MYH7 and 1 MYBPC3)

**Conclusion.** Mutations were identified in 70% of families with HCM, with a high detection of 93% in those with a family history of sudden cardiac death but only 40% in sporadic cases. The turn around time for results from the index case is now down to around 3 months making cascade screening efficient and realistic in patients with HCM. This is not the case yet in FDCM and a reassessment of the genes to be screened is underway.

#### P16.09

##### Novel desmocollin-2 gene mutation associated with arrhythmogenic biventricular cardiomyopathy

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**Introduction:** Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a frequent cause of sudden death. The disease is usually caused by mutations in desmosomal genes (desmoplakin, desmocollin-2, desmoglein-2, plakophilin-2 and plakoglobin), which have been associated with left ventricular involvement.

**Material and methods:** Clinical, familial and genetic (direct sequencing of five desmosomal genes) study of a Spanish group of no related patients with ARVC was done.

**Results:** A novel splice site mutation in intron 11 of desmocollin-2 gene (IVS11+2T>C) was detected in a 44 year old ex-professional pole-vault-jumper who had suffered a cardiac arrest while jogging. This change was absent in 100 caucasian healthy controls and a truncation of the protein in exon 11 was predicted. His baseline electrocardiogram showed negative T-waves in right precordial leads. The echocardiogram showed left ventricle dilation (56 mm) and systolic dysfunction (ejection-fraction:45%), and mild global right ventricle dilation with hypo- and akinetic areas in the outflow tract. A cardiac defibrillator was implanted and a single appropriate intervention was recorded five years later. At family screening, the mutation was found in his asymptomatic 70 year old father, who showed left ventricular dilation (60 mm) with systolic dysfunction (ejection-fraction:50%), and mild right ventricular dilation with doubtful areas of hypertrabeculation and hypokinesia.

**Conclusions:** Only 5 ARVC related mutations have been previously described desmocollin-2. Most of them have left ventricular involvement, as we found in our two carriers. Competitive sport practice could have contributed to an earlier and more severe disease expression with malignant ventricular arrhythmias. Desmocollin-2 gene mutations could early affect left ventricle.

#### P16.10

##### Sporadic arrhythmogenic right ventricular cardiomyopathy due to a de novo mutation

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We report the case of a 41-year-old man with a diagnosis of sporadic arrhythmogenic right ventricular cardiomyopathy (ARVC). The diagnosis fulfilled the International Task Force diagnostic criteria with extensive T-wave inversion on the electrocardiogram (ECG), late potentials

on the signal-averaged-ECG and typical abnormalities of the right ventricle on cardiac imaging. We documented right ventricular arrhythmia with frequent spontaneous ventricular ectopies and fast ventricular tachycardia with left-bundle-branch block morphology induced by the electrophysiological study. The genetic screening of the four desmosomal genes known to be involved in ARVC (plakophilin-2, desmoplakin, desmoglein-2 and desmocollin-2) identified the heterozygous missense mutation R49H in the desmoglein-2 gene. This mutation is located in the highly conserved cleavage motif RXK/RR that is recognized by pro-protein convertases and is thus predicted to prevent efficient pro-desmoglein-2 maturation. The mutation was absent in both parents, and we demonstrated that it was a de novo mutation. To our knowledge, this is the first description of a de novo mutation in ARVC. Appearance of a de novo mutation in the desmoglein-2 gene (that is an essential component of desmosome that mediates cell-to-cell adhesion) provides compelling genetic evidence for the involvement of this gene in ARVC. The recognition of de novo mutations has important implications, including for clinical practice, since individuals with sporadic ARVC caused by a de novo mutation can transmit the disease gene to 50% of their offspring. This suggests that the benefit of molecular genetics can be extended to sporadic ARVC, and may improve genetic counselling.

#### P16.11

#### Implications of consanguinity in families with hypertrophic cardiomyopathy

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Introduction: By presenting two cases, we illustrate the implications of consanguinity in families with hypertrophic cardiomyopathy (HCM), with respect to patient care and DNA-analysis techniques.

Case reports: Case 1: A man with HCM due to the c.2609G>A mutation in MYH7 died suddenly at age 43. His wife was asymptomatic, but because she was a second cousin of the proband, we performed DNA-analysis on her. She carried the same mutation. DNA-analysis in their asymptomatic 5 year-old son showed homozygosity for this mutation. At cardiologic exam, he had severe signs of HCM and is now carefully followed at a paediatric cardiology department.

Case 2: The son of an asymptomatic consanguineous couple (first cousins) had a myectomy because of obstructive HCM at age 10 and ICD-implantation at age 17 because of ventricular tachycardia. Using DHPLC, no mutations were identified in MYH7, MYBPC3 and TPM1. Because this boy had the same family name as the man in case 1, we performed sequence analysis to see if the c.2609G>A mutation in MYH7 could be detected. The mutation was found to be present in homozygous state, which had been missed by DHPLC.

Conclusion: These cases illustrate that in consanguineous families with HCM, presence of a pathogenic mutation in both persons of a couple must be considered, with the subsequent possibility of homozygosity in their children. (Presymptomatic) DNA-analysis is recommended for at risk consanguineous partners and young children who are at risk for homozygosity. Mutation analysis techniques that allow the detection of homozygous mutations must be used.

#### P16.12

#### Geno-phenotype characterisation of hypertrophic cardiomyopathy patients that evolve through end-stage heart failure

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Heart transplantation (HTx) is the sole therapeutic option for selected patients with hypertrophic cardiomyopathy (HCM) and congestive heart failure (CHF). We aimed at determining the prevalence and the outcomes of a consecutive series of genotyped patients diagnosed with HCM with a dilatative or restrictive evolution. The clinical series is constituted of 146 unrelated probands diagnosed with HCM and 244 genotyped relatives. HCM was diagnosed according to the WHO criteria. Probands and relatives underwent genetic testing after genetic counselling and written consent. Of 390 genotyped individuals, 280 were affected and 110 healthy carriers. The mean age of affected members was significantly higher compared to those of the healthy

carriers ( $34.8 \pm 17.2$  vs  $28.5 \pm 16.3$  years,  $p=0.004$ ). The molecular genetic analysis identified mutations in one of the following disease genes: MYH7 (n=104, 27%), MYBPC3 (n=177, 45%), TNNT2 (n=25, 6%), TNNI3 (n=22, 6%), LAMP2 (n=3, 0.5%), PRKAG2 (n=3, 0.5%), tCAP (n=8, 2%), MYOZ1 (n=2, 1%), mtDNA (n=15, 4%). Patients with Anderson-Fabry disease were excluded. 31 patients (8%) were found to carry a compound or double heterozygosity. After  $90 \pm 70$  months, 76 (27%) affected patients had dilatative (n=51) or restrictive (n=25) evolution. Among the former 51 patients, 38 (74.5%) had one of the following events: CHF death while awaiting for HTx (n=10); HTx (n=13); appropriate ICD intervention plus HTx (n=5); sudden cardiac death or appropriate ICD intervention (n=10). One fourth of genotyped HCM patients developed dilatative or restrictive evolution. The HCM that evolves through dilatation show more HF-related events than those that evolve through restrictive hemodynamics ( $p=0.004$ ).

#### P16.13

#### Phenotypic characterization of hypertrophic cardiomyopathy associated with K600fs mutation in cardiac myosin-binding protein C gene

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Introduction: MyBPC3 mutations are the most frequent causes of hypertrophic cardiomyopathy. A high percentage of these mutations are frameshift. K600fs was previously reported in only one French patient and is predicted to encode a truncated peptide, that may be unable to be incorporated into sarcomere A-bands.

Methods: Clinical study and phenotypic characterization of probands and family members of eight Spanish families where K600fs was detected.

Results: We found 21 carriers in 8 families from a region of Galicia. Penetrance >90% in >30 years and cosegregation was found in all families. Mean maximal wall thickness was 20mm. Morphology of hypertrophy and late enhancement localization at cardiac MRI was reproducible in several families. Carriers were in NYHA-II and left ventricular outflow tract obstruction was present in 4 patients. Left atrium dilation and early atrial fibrillation (AF) were present in 9 and 6 carriers, respectively. Surgical myectomy was done in 1 carrier and automatic defibrillators were implanted in 2 (primary prevention at 26 and 37 years). Two sudden deaths (18 and 42 years) and one cardiac transplant (35 years) were reported in young family members. Stroke related death was reported in two young carriers (41 and 55 years).

Conclusions: Correct familial and clinical evaluation in suitable number of mutation carriers allow us to establish genotype-phenotype correlations, which are difficult when the number of cases reported is limited. K600fs mutation could have a common founder effect in Galicia. Mutation carriers develop left ventricular hypertrophy at young age and main complications are AF and strokes.

#### P16.14

#### Investigation of polymorphisms in non-coding region of human mitochondrial DNA in 31 Iranian Hypertrophic Cardiomyopathy (HCM) Patients

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The D-loop region is a hot spot for mitochondrial DNA (mtDNA) alterations, containing two HyperVariable Segments, HVS-I and HVS-II. In order to identify polymorphic sites and potential genetic background accounting for Hypertrophic CardioMyopathy (HCM) disease, the complete non-coding region of mtDNA from 31 unrelated HCM patients and 45 normal controls were sequenced. The sequences were aligned upon the revised Cambridge Reference Sequence (rCRS) and any incompatibilities were recorded as numerical changes in homoPolymeric C Tract (PCT), single base substitutions (SBS), insertions and deletions (Indels). Nucleotide substitutions were found to make up the majority of the mutations, rather than indels. We drew significantly high transition rate (81.8%) versus lower frequency of transversions (18.2%). 12 polymorphisms were identified in this study which had not been published in the MitoMap database. PCT changes at position 303-309 were detected in 83% of our samples. Our results suggest that an increased level of HVS-I and HVS-II substitutions may be an

indicator of mitochondrial DNA instability. Furthermore, mtDNA mutations may play an important role in pathogenesis of cardiac arrest which has remained unexplained for long.

#### P16.15

#### Possible role of accumulation mtDNA mutations in LQTS Patients

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<sup>3</sup>Department of Genetics, Science School, Tarbiat Modares University (TMU), Tehran, Iran, <sup>4</sup>Tehran, Islamic Republic of Iran, <sup>5</sup>Heart Arrhythmia Center, Tehran, Islamic Republic of Iran, <sup>6</sup>Special Medical Center, Tehran, Islamic Republic of Iran, <sup>6</sup>Department of Medical Chemistry, Medical University, Vienna, Austria. Syndrome of Long QT (LQTS) is among arrhythmia disorders of the heart that causes of sudden cardiac death in young individuals. As yet, most of investigations have focused on nuclear genome for finding of genetic defects in this disorder, but some of the cases with LQTS cannot be explained by mutations of identified genes. On the other hand, it has been reported that the activity of ion channels in cardiomyocytes is sensitive to ATP level. It prompted us to focus on the mitochondrial DNA and monitor the point mutations of genome which are probably the cause of respiratory chain defects and reduced ATP generation. We searched about 55% of the mitochondrial DNA (mtDNA) by temporal temperature gradient gel electrophoresis (TTGE) and DNA fragments showing abnormal banding patterns were sequenced for identification of exact mutations. In 39 patients (33 familial and 6 sporadic cases), for the first time, we detected 35 mtDNA mutations which 8 were novel (23%) and 27 (77%) have been reported in other mitochondrial diseases. Our results showed that these mutations in LQTS patients were higher than normal controls ( $P<0.0001$ ) and number of mutations in LQT patients with syncope is higher than patients without syncope ( $P<0.001$ ). As the mitochondrion's ATP synthesis is important in heart, it is possible that mutations and their accumulation in mtDNA could constitute a predisposing factor that in combination with environmental risk factors may trigger the arrhythmia disorders and may be the link between this syndrome and dysfunctions of mitochondria.

#### P16.16

#### A possible new Dutch founder in three families with hypertrophic cardiomyopathy, a more malignant phenotype of the MYL2 mutation E22K

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Familial hypertrophic cardiomyopathy (HCM) is a genetic cardiac disorder, which affects 1 in 500 subjects. HCM is characterized by left ventricular hypertrophy, arrhythmias and sudden death. Mutations in the Myosin Light Chain 2 gene (MYL2) are rare (4%) and disease phenotype is described as mild. We present three large Dutch families with MYL2 mutation E22K to demonstrate that the phenotype does also include severe hypertrophy and sudden death at a young age. DNA-analysis in 11 siblings of family 1, 11 siblings of family 2, and 2 of family 3 showed an E22K mutation (c.64G>A) in MYL2. Other sarcomeric genes were analysed and no mutations were found. There were 2 sudden deaths in family 1. In family 2 the index person had severe HCM and ICD. In family 3 sudden death was reported. The families reported in literature with the MYL2 mutation E22K showed late onset moderate septal hypertrophy with benign disease course and good prognosis. Our families the disease showed a far more malignant character. Because of the rarity of MYL2 mutations, haplotype analysis of the MYL2 region was performed to link the three families. The families shared a similar haplotype implying they are related. E22K might therefore be a founder mutation. We'll perform genealogy to establish the relationship between the three families to calculate the family tree mortality ratio of this mutation. These findings are relevant in the counseling and treatment of other patients carrying this mutation.

#### P16.17

#### Immunohistochemical analysis of TGF-beta receptor 1 and mutation screening of TGFBR1 gene in patients with different types of cardiomyopathy

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Transforming growth factor beta receptor I (TGFBR1) belongs to the transmembrane-spanning protein serine/threonine (Ser/Thr) kinases. TGFBR1 activates numerous signaling pathways by autophosphorylation and downstream signaling proteins on specific Ser/Thr residues. TGFBR1 involved in several cellular processes, including growth inhibition, apoptosis, proliferation, and extracellular matrix production. Mutations and abnormal expression of the TGFBR1 genes have been associated with some cardiovascular diseases and several human cancers as well as TGF-beta 1 resistance. Our previous immunohistochemical data showed hyperexpression level of TGFBR1 in myocardium in some individuals with hypertrophy of left ventricular (post-mortem study). We suppose that altered by mutations TGFBR1 receptors may involve in development of some types of cardiomyopathies. We have screened half of TGFBR1 gene in groups of patients with dilated cardiomyopathy (ejection fraction 20-45%; n = 83) and severe left ventricular hypertrophy (wall thickness more than 1,5 mm; n=118). We found one missense-mutation c.457G>A (V153I) in third exon in one patient with dilated cardiomyopathy. Mutation V153I affects transmembrane domain of TGF-beta receptor I and therefore may alter the localization of the receptor in cell membrane. Screening mutations of TGFBR1 gene continues.

#### P16.18

#### Survey of 6300 Finns for desmosomal gene mutations associated with risk of arrhythmogenic right ventricular cardiomyopathy

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Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a severe arrhythmic disorder, mainly caused by dominant mutations of the desmosomal cell adhesion proteins, including plakophilin-2 (PKP2), desmoplakin (DSP), desmoglein-2 (DSG2) and desmocollin-2 (DSC2). Upon search of 29 Finnish ARVC probands, we have previously identified two apparently disease-causing mutations (Q59L and N613K) and one possibly disease-modifying variant (Q62K) of PKP2. As an extension of these studies, we screened our ARVC probands for three other desmosomal genes and conducted a search for ARVC-associated mutations in a large Finnish population sample (Health 2000 Study, n = 6300). A novel frameshift mutation (E1020fsX1037) of DSG2 appeared in one ARVC proband, and another patient carried a DSP variant T1373A, whereas DSC2 was not mutated in any of the 29 patients. Ultrastructural changes compatible with ARVC were seen in endomyocardial biopsies of two patients: one with mutation DSG2 E1020fsX1037 and another with both PKP2 N613K and Q62K. In the population sample, a total of 31 subjects (0.5%) carried a desmosomal gene mutation, PKP2 Q59L being the most prevalent form (n = 19). Mutation carriers showed only limited phenotypic characteristics as judged by ECG recordings. Preliminary analyses suggest that heart failure and ventricular arrhythmias are slightly more prevalent in PKP2 Q59L carriers than in non-carriers. In conclusion, up to 1 in 200 Finns carry a mutation with a proposed risk of ARVC. However, our findings suggest that penetrance of a typical ARVC phenotype may require additional (genetic or nongenetic) factors, in addition to the mutant desmosomal gene itself.

**P16.19****Genetic screening of long QT syndrome (LQTS) in Sweden**

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**Background:** Long QT syndrome (LQTS) is a hereditary cardiac disease characterized by prolongation of the QT interval on ECG and presence of syncope, seizures, and sudden death. The most common genes implicated in LQTS are the cardiac ion channel subunits *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1* and *KCNE2*, accounting for about 70% of the identified mutations in patients with a clinical diagnosis. The symptoms are very variable in LQTS patients, and genotype influences the clinical course.

**Objective:** This study aims to report the spectrum of LQTS mutations in Sweden, as well as genotype-phenotype correlations. Knowledge of founder mutations or certain subdiagnostic criteria for different mutations will facilitate the molecular genetic analysis, thereby reducing the turn-around time and cost.

**Methods:** Two hundred consecutive, unrelated patients referred for LQTS genetic testing will be evaluated. Coding sequences and splice sites of the five genes, as well as alternate transcript exon 1B of *KCNQ1* and *KCNH2*, will be screened for genomic variants by denaturing high-performance liquid chromatography (dHPLC) and DNA sequencing. Furthermore, multiplex ligation-dependent probe amplification (MLPA) will be performed in all patients to detect large deletions or duplications. Selected patients will also be screened for mutations in *RYR2*, a gene known to be involved in the clinically overlapping disease catecholaminergic polymorphic ventricular tachycardia (CPVT1).

**Results:** So far, a total number of 180 probands have been included in the study. Preliminary results have identified two common founder mutations, as well as other mutations in about 63% of the patients, whereas double mutations seem to be rare.

**P16.20****SCN5A mutations in Brugada Syndrome Spanish patients**

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Brugada syndrome (BS) is characterized by cardiac ST-segment abnormalities and a high risk of ventricular arrhythmias and sudden death. Age at diagnosis ranges from 2 days to 85 years. BS is diagnosed based on clinical findings, and *SCN5A* ( $\alpha$ -subunit of the sodium channel), is the only gene currently known to be associated with Brugada syndrome. Sequencing of *SCN5A* identified mutations in approximately 25% of individuals with BS. Mutation carriers are frequently asymptomatic, even at advanced ages. The identification of asymptomatic mutation carriers enables the use of preventive measures to avoid ventricular arrhythmias. Most of the *SCN5A* mutations are private, and the 26 coding exons should be analysed to define the mutational status of BS patients.

We sequenced this gene in 25 BS patients from the region of Asturias (Northern Spain) (81% male; mean age at diagnosis,  $41 \pm 14$  years, range 17-66). The 26 coding exons were PCR-amplified and sequenced using BigDye chemistry in an ABI3130 system. All the variants found in the patients were also screened in 200 healthy population controls, using SSCA and DHPLC.

We found 17 *SCN5A* variants, and 13 were also found in the controls, thus being DNA polymorphisms. Four were putative mutations, found only in one patient and none of the controls: three missense changes (Ala2Thr; Ala739Thr; Val134Ile) and a splicing mutation (IVS18-1G>A, intron 18).

We found relatives of these patients who were mutation carriers, some of them symptomatic. We discussed these findings, in particular the genetic counseling of *SCN5A* mutation carriers.

**P16.21****A novel alpha-tropomyosin mutation in a large family with dilated cardiomyopathy**

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Dilated cardiomyopathy (DCM) is characterized by dilatation and systolic contractile dysfunction of the left and/or right ventricle and consequently by an impaired systolic function. The origin of DCM is heterogeneous but genetic transmission of the disease accounts for 10-35% of cases. We present a large three-generation family in which DCM inherits as an autosomal dominant trait. Six family members have DCM, with the age of diagnosis ranging from five months to 52 years. The youngest one was initially diagnosed with non-compaction cardiomyopathy (NCCM) and died at the age of five. Three additional young children died of suspected heart problems. We mapped the phenotype of this family to chromosome 15 and subsequently identified a missense mutation in alpha-tropomyosin (TPM1), leading to a p.D84N amino acid substitution. Tropomyosins are sarcomeric, thin filament proteins that play fundamental, structural and regulatory roles in skeletal and cardiac muscle cells. The far majority of mutations in TPM1 are associated with hypertrophic cardiomyopathy (HCM) and few with DCM. Our mutation has not been described before and was not detected in 400 control chromosomes. It co-segregates with all clinically affected family members, and is predicted, using existing atomic and protein models, to weaken the binding of tropomyosin to actin. In conclusion, DCM causing mutations in TPM1 are associated with a diverse phenotype including also lethal, early onset forms. The screening of patients and families with these forms of DCM for TPM1 is therefore warranted.

**P16.22****Investigation of human mitochondrial DNA in Iranian Hypertrophic Cardiomyopathy (HCM) patients**

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Mitochondrial (mt) DNA defects, both deletions and tRNA point mutations, have been associated with cardiomyopathies. The aim of the study was to determine the mtDNA mutations in Hypertrophic cardiomyopathy (HCM) Iranian patients.

Hypertrophic cardiomyopathy (HCM) is widely accepted as a pluricausal or multifactorial disease. Because of the linkage between energy metabolism in the mitochondria and cardiac muscle contraction, it is reasonable to assume that mitochondrial abnormalities may be responsible for some forms of HCM. We analysed the whole mitochondrial genome in a series of 31 patients with HCM for alterations and compared the findings with those of 30 control subjects. A total of 16 sequence changes could be identified. These sequence changes were distributed among the whole mitochondrial DNA (mtDNA). An increased number of novel missense mutations could be detected nearly in all genes encoding for protein subunits in HCM patients subjects. Four mutations were found that are unpublished. The c.4384T>C in **tRNA glutamin**, c.9063A>G in **ATPase6**, c.2071 T>C, c.3170C>A, in noncoding MTRNA2 16S. Also 33 polymorphisms were identified in this study which had not been published in the MitoMap database. The c.16189T>C mutation in the D-loop region that is associated with susceptibility to DCM could be detected in 3% of patients as well as in 0% of controls. Furthermore, mtDNA mutations may play an important role in pathogenesis of cardiac arrest which has remained unexplained for long.

**P16.23****Severe hypertrophic cardiomyopathy in adults and children: similar gene mutations for a wide spectrum of clinical manifestations**

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HCM is a myocardial disease characterized by huge phenotypic and genotypic heterogeneity. Affecting 1 in 500 individuals, it's the most common cause of sudden death in young athletes. HCM is caused by mutations in at least 13 genes, most commonly MYH7 ( $\beta$ -myosin heavy chain) and MYBPC3 (myosin-binding protein C). We analysed these genes in 105 patients: 41 with HCM diagnosed before 18 years of age (group A: mean age, 7 years) and 64 adults (group B: mean age, 52 years). From 2001 to 2006 the analysis was performed by

SSCP or DHPLC and then by sequencing. Forty-two patients (40%, 8 group A, 34 group B) underwent surgical miectomy, 32 (30%, 19 group A, 13 group B) had heart failure requiring transplantation, 8 (8%, 6 group A, 2 group B) died suddenly and 23 (22%, 8 group A, 15 group B) were outpatients.

We identified 21 mutations in group A (51%) and 24 in group B (38%). The detection rate for SSCP/DHPLC was 21/59 mutations (36%), while by sequencing was 24/46 (52%). Twenty-five mutations were not previously reported. Four variants occurred in group A (10 patients) and group B (4 patients). They were associated with different outcomes and age at onset.

No significant differences in genetic causes were found between childhood- and adulthood-onset HCM or between patients with diverse outcomes. Although most patients had a severe HCM that could explain this lack of differences, a wide genome approach should be useful to detect potential genetic modifiers.

#### P16.24

#### Complex sarcomeric genetic status is not an important modifier of disease severity in *MYBPC3* associated hypertrophic cardiomyopathy

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**Background:** Hypertrophic cardiomyopathy (HCM) is a genetically heterogeneous disorder with a high degree of inter- and intrafamilial variability in clinical expression. Mutations in more than 11 genes, mostly encoding sarcomeric proteins, are known to cause HCM. Variability in clinical expression is thought to be caused by the action of currently unknown modifying factors. Current consensus partially explains the variability in clinical expression by the effect of additional, secondary, mutations in sarcomeric genes.

To analyse whether a complex HCM genotype is an important modifier of disease severity in HCM we completely analysed 11 HCM genes in a large cohort, homogeneous with respect to primary HCM causing mutation.

**Methods:** We analysed the complete coding regions of *MYH7*, *MYBPC3*, *MYL2*, *MYL3*, *TNNI2*, *TNNI3*, *TNNC1*, *ACTC1*, *TMP1*, *TCAP* and *CSRP3* in a large cohort of patients with a truncating *MYBPC3* mutation as primary HCM defect. The patients from the cohort were clinically diagnosed as either having a 'mild' (no cardiac complaints, IVS<20mm) or a 'severe' phenotype (myectomy, HTX, (aborted) sudden cardiac death, necessary ICD implantation, cardiac related stroke, IVS30mm).

**Results:** no additional mutational "burden" was seen in the group with severe HCM compared to the group with milder HCM.

**Conclusion:** Contrary to general consensus, the severity of phenotypic expression of HCM is not primarily dependent on the modifying effects of secondary sarcomeric mutations. Disease prognosis and severity in HCM is more likely to be modified by environmental factors as well as genetic factors, other than additional mutations in the analysed sarcomeric and Z-disk genes.

#### P16.25

#### Diagnostic mutation analysis in hypertrophic cardiomyopathy by DNA resequencing array

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Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiac disease (1/500) characterized by a remarkable clinical and genetic heterogeneity. More than 450 different pathogenic mutations in at least 20 genes have been identified so far, which makes molecular

analysis by classical methods very time-consuming and expensive. Mutation detection for HCM has a growing impact on the medical management of patients/families. We have developed a 30 Kbp HCM-DNA-resequencing-array (CustomSeq Affymetrix) for all exons (n=160), splice-sites and 5'-UTR of 12 HCM genes (Fokstuen et al. 2008). This HCM-array, which we currently use in clinical practice is very efficient to detect single nucleotide substitutions accounting for up to 86% of all HCM mutations. Actually it does not detect small indels. We analysed 115 patients from 5 different centres. Overall, we identified 30 different single nucleotide substitutions in the coding regions or splice sites of *MYH7*, *MYBPC3*, *TNNI2*, *TNNI3*, *TPM1* and *MYL3* in 42 patients (37%). Twelve variants were reported as known mutations and 14 were novel changes not found in a control population study (>200 chromosomes). Furthermore, we identified 4 known SNPs/variants previously reported as mutations for HCM. Our DNA resequencing array appears to date as the most rapid and cost-effective technology for mutation screening in HCM. Further improvement of the software may detect small insertions/deletions in the future. The HCM-array provides a first attempt of high throughput sequencing methodology which will further develop and probably become the method of choice for routine molecular diagnosis of heterogeneous disorders such as HCM.

#### P16.26

#### Haploinsufficiency of *MYBPC3* as the genetic cause of sarcomeric hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM, OMIM#192600) is an inherited myocardial disease that shows a large genetic and allelic heterogeneity. Over 300 mutations spread among several genes encoding myofilament, calcium-handling and mitochondrial proteins have been identified. The most common form is sarcomeric HCM, with hundreds of disease-associated mutations found in more than 8 genes. Mutations in *MYH7* ( $\beta$ -myosin heavy chain) and *MYBPC3* (myosin-binding protein C) are responsible of about 60% of all HCM cases. The observation that most variants found in sarcomeric genes are missense mutations led to the proposal of dominant negative action as the pathogenic mechanism. Instead, *MYBPC3* point mutations generate frequently truncated protein suggesting haploinsufficiency as the cause of the disease. We hypothesized that larger gene rearrangements not detectable by sequencing may be found in HCM patients. We analyzed *MYBPC3* gene by MLPA in 36 patients referred to our laboratory with clinical and histological diagnosis of HCM. All patients were negative for *MYBPC3* and *MYH7* point mutations. We found in 1 patient a partial deletion spanning from exon 28 to 34 of *MYBPC3*. This patient was a 31 years old male with an obstructive HCM, requiring surgical miectomy at the age of 25. Similar severe clinical manifestations were found in patients with *MYBPC3* missense mutation leading to truncated proteins. The observation that the phenotype of this patient is indistinguishable from those of patients with point mutations supports the hypothesis that haploinsufficiency is the mechanism at the basis of *MYBPC3*-associated HCM.

#### P16.27

#### Genetic diagnostic of hypertrophic cardiomyopathy using mass spectrometry arrays and high resolution melting

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Hypertrophic Cardiomyopathy (HCM), a relatively common genetic myocardial disorder (about 1:500), is the most frequent cause of sudden death in young athletes. This autosomal dominant genetic disease, caused frequently by mutations in sarcomeric genes, is characterized by a hypertrophied, non-dilated left ventricle. Genetic testing of HCM-patients, valuable for diagnosis, is hampered by the multiplicity of genes (20) and mutations (609) involved. Available genetic diagnostic services consist in the analysis of only the most frequently mutated genes, failing to detect a mutation in 1/3 of the probands. Coupling two high-throughput techniques, Mass Spectroscopy Genotyping (MSG) and High Resolution Melting (HRM), enabled us to study all the 20 HCM-associated genes, thus finding mutations in *CSRP3* (missed

in common analysis) and in *MYBPC3*(novel). The patient group consisted of 16 individuals with a clinical diagnostic of HCM. DNA extraction was extracted from peripheral blood. All samples from the patient group were genotyped by MSG using a MassARRAY MALDI-TOF. The samples with no mutation found were scanned for novel mutations by HRM. All variants found were confirmed by automatic sequencing. The *CSRP3* c.128delC mutation was found in heterozygosity in a 50-year old patient with diffused left ventricle hypertrophy. A novel mutation of the *MYBPC3* was discovered in another HCM patient: c.C>T817/p.Arg273Cys. Although still uncharacterized as disease-causing, affects a codon known to harbor a known HCM-causing mutation - p.Arg253His. This coupling of innovative techniques allowed the detection both of a known mutation on a seldom analyzed gene and a probably HCM-causing novel mutation.

#### P16.28

#### Familial noncompaction cardiomyopathy: genetic and cardiologic features in adults and children

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**Background:** Noncompaction cardiomyopathy (NCCM) features a thickened bilayered left ventricular wall with a thin, compact epicardial layer and a thick endocardial layer with prominent intertrabecular recesses. NCCM is genetically heterogeneous. Similarly to hypertrophic (HCM) and dilated cardiomyopathy (DCM), NCCM has been associated with mutations in sarcomere genes. In order to contribute to a genetic classification for NCCM a systematic cardiologic family study was performed in a cohort of 56 consecutively diagnosed and molecularly screened patients with isolated NCCM (47 adults and nine children).

**Methods and Results:** Cardiologic screening with electrocardiography, echocardiography and physical examination of 169 relatives from 46 unrelated NCCM probands revealed familial cardiomyopathy in 34 families (74%), including NCCM, HCM and DCM. Seventy-four percent of the relatives newly diagnosed with cardiomyopathy were asymptomatic, explaining that 54% of familial disease remained undetected by ascertainment of family history prior to cardiologic screening. The molecular screening included analysis of 17 genes yielding 29 different mutations in 23 probands (41%); 18 adult and five children. Fifteen single mutations and one double mutation on the same allele were transmitted in an autosomal dominant mode. Six adults and two children were compound or double heterozygous for two different mutations. In 19/34 (56%) of familial NCCM the genetic defect remained unknown.

**Conclusion:** NCCM is predominantly a genetic disorder, requiring genetic counseling, DNA diagnostics and, also in absence of a genetic cause, cardiologic family screening.

#### P16.29

#### Takotsubo and congenital LQTS in a patient with a novel mutation in the *KCNH2* gene

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Delayed cardiac repolarization may be caused by inherited mutations in cardiac ion channel gene subunits (congenital long QT syndrome, LQTS) or secondary to cardiac pathologies, including the recently-described Takotsubo cardiomyopathy.

We report the case of a female patient admitted at the emergency ward of the Lausanne university hospital because of a sudden convulsive syncopal event. The observation of a transient alteration of the ECG repolarization with a torsades de pointes episode 9 days after a surgical stress suggested a Takotsubo cardiomyopathy. After transient normalization of the ECG, congenital LQTS was diagnosed on the basis of a persistent QT prolongation. Genetic analyses identified a 7 amino-acid duplication (nc.343-363dup) located in the PAS domain of hERG1 N-terminus in the gene *KCNH2* coding for the hERG1 channel.

This mutation has not been reported yet.

Three recent case reports suggested that both LQTS and Takotsubo syndrome may be causally related but no mutation in the LQTS genes have been identified yet. In conclusion, we describe a patient with Takotsubo uncovering a congenital form of LQTS. We show that LQTS is caused by an unusual *KCNH2* mutation that has been characterized at the biophysical and biochemical levels. A putative causal relationship between LQTS and Takotsubo is discussed. However, the mechanisms underlying the link between the two disorders, if any, remain to be investigated.

#### P16.30

#### Does δ-sarcoglycan-associated autosomal dominant cardiomyopathy exist?

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The sarcoglycans are part of the dystrophin-glycoprotein-complex (DGC), an oligomeric complex spanning the plasma membrane of skeletal and cardiac muscle fibres. The sarcoglycan deficient limb girdle muscular dystrophies (LGMDs) are characterized by progressive weakness of the pelvic and shoulder girdle musculature and affected patients often develop a progressive and potentially fatal dilated cardiomyopathy (DCM). Dominant inheritance has not been reported in sarcoglycan-deficient LGMD. However, a previous report described autosomal dominant mutations in the δ-sarcoglycan (*SGCD*) gene in patients with familial and sporadic cases of DCM without significant skeletal muscle involvement. Here we clinically and genetically characterize a consanguineous family with a homozygous novel missense mutation (p.A131P) in the *SGCD* gene and a second δ-sarcoglycan mutation that has previously been reported to cause a fatal autosomal dominant DCM at young age. This second heterozygous mutation (p.S151A) was found in 4 heterozygous carriers for the A131P mutation, aged 3 to 64 years. Comprehensive clinical and cardiac investigation in all of the compound heterozygous family members revealed no signs of cardiomyopathy or LGMD. Even in the presence of a second disease causing mutation, the p.S151A mutation in the *SGCD* gene does not result in cardiomyopathy. This finding questions the pathological relevance of this sequence variant for causing familial autosomal dominant DCM and thereby the role of the *SGCD* gene in general as a disease causing gene for autosomal dominant DCM.

#### P16.31

#### Blood pressure changes associated with eNOS gene polymorphism at patients with stress cardiomyopathy

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**Objective:** Possibility of pathological changes in myocardium caused by acute stress influence is well-known at this moment. At the same time chronic psychoemotional stress (PES) could be an independent reason of stress cardiomyopathy (SKMP) at the high-risk professional group's person, which may have genetic markers of «low stress resistance», associated with vascular reactions caused by PES. We investigate blood pressure (BP) level and endothelial NO-synthase gene (eNOS) polymorphism at the persons exposed to chronic professional PES' influence and also with noncoronarogenic heart' damages.

**Design and methods.** The subjects of research were railway' engine-drivers: 58 men with SKMP diagnosed in case finding clinically significant arrhythmia and electrical conductivity disturbance at 24-h ECG monitoring and also non-ischemic repolarization abnormalities on ECG and/or ECG stress test. Exception criteria were coronary disease, arterial hypertension (above 140/90 mmHg), Primary/secondary cardiomyopathy other genesis, including inflammatory compared with 78 men without a cardiovascular pathology. All patients underwent clinical BP measurement and analysis for 4a/4b eNOS gene polymorphism by PCR.

**Results.** The systolic (SBP) and diastolic (DBP) blood pressure levels at 4a (genotypes 4a/4b and 4a/4a) SKMP patients were signifi-

cant increased in comparison with 4b homozygous SKMP patients ( $123,07 \pm 8,3$  vs  $128,9 \pm 7,2$  mm Hg,  $P < 0,01$ ;  $78,8 \pm 5,7$  vs  $82,4 \pm 5,6$  mm Hg,  $P < 0,02$ ). There wasn't any significant difference SBP and DBP levels in 4a and 4b/4b patient in control.

Conclusions. 4a allele eNOS gene associated with increased SBP and DBP levels at engine-drivers with SKMP that support the existence of genetic predisposition of vascular reactions caused by emotional stress.

#### P16.32

#### Scapuloperoneal syndrome with facial weakness in Russian family with 4q35 linkage

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Facioscapulohumeral muscular dystrophy (FSHMD), is a dominantly inherited, late onset, heterogeneous neuromuscular progressive disease. There is considerable clinical variability, even within families. 39 members of a big family with segregation disease in 3 generation from Central region of Russia were investigating. 14 patients have been revealed by the clinical and EMG examination were revealing. Genomic DNA was isolated, using standard procedures, from peripheral leukocytes. Linkage analysis was performed with D4S1523, D4S2930 markers from 4q35 and D10S212, D10S1711 from 10q26 regions. The FSHD 10q26 loci were excluded by the result of linkage analysis.

The clinical picture is submitted with an atrophy and weakness m.m. orbicularis oris, rhomboideus major, supraspinatus, pectoralis major, gluteus maxima, quadriceps, and foot dorsiflexors. M.m. orbicularis oculi, biceps brachii, latissimus dorsi are not involved in pathological process. A debut of disease in the age of  $26 \pm 3,7$  with dissymmetric defeat m.scapular or m.orbicularis oris with slow progressing process and long preservation of impellent functions. The degree of atrophies and the list of the struck muscles considerably varied at separate patients. In the unwrapped stage of disease was observed infringement of gait on type "steppage". Feature of clinic at all patients were preservation tendinous reflexes and absence of sensitive infringements. Established moderately expressed myopathic pattern were observed by the needle EMG examination. Motor nerve conduction velocity upper and lower limbs are normal. Serum CK level was normal at all patients.

The addition of received clinical-molecula-genetic data of the submitted family allows relating facioscapuloperoneal syndrome to the FSH-MD 1B variant.

#### P16.33

#### Prenatal diagnosis of the Charcot-Marie-Tooth disease type I in South-West Siberia region of Russia

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CMT1 is the major form of Charcot-Marie-Tooth disease. More than 70% cases of CMT1 are associated with 1.5-Mb duplication in 17p11.2-region containing *PMP22* gene - CMT1A form; CMTX caused by *Cx32* gene mutations is the second most common form. We present here our first experience in prenatal diagnosis (PD) for CMT in families at risk pregnancies: three cases - with 17p11.2-duplication, and one case - with *Cx32* gene point mutation.

The most effective strategies for CMT1A molecular diagnosis is based on PCR-analysis of microsatellite STRs. We estimated the diagnostic potential of five STR-markers within the 17p11.2: D17S2224, D17S2226, D17S2227, D17S2228, D17S2230 in Russian Siberian population, and observed heterozygosities were 0.730, 0.826, 0.763, 0.626, and 0.908. This STR-panel was greatly informative in Russians and was applied for analysis of 17p11.2-duplication in prenatal DNA-diagnosis allowing very fast and reliable results, and even gene dosage reliable interpretation is available with this STR-marker panel. In three CMT-families with 17p11.2-duplication we predicted two healthy and one affected fetus.

In CMTX-family gene mutation in *Cx32* previously was detected by SSCP-analysis and DNA-sequencing: point nucleotide mutation C490T (Arg142Trp). In case of C490T-mutation Mspl-site is lost, and we used restriction analysis for prenatal study for woman which was

showed is heterozygosity for C490T-mutation.

This is the first experience in PD for CMT in Russians families in South-West Siberia region. As conclusions we can accentuate that PD requires family mutation should be known previous to PD, so, prenatal analysis requires the DNA study in family before the prenatal procedure.

#### P16.34

#### A novel missense mutation in the *GJB1* gene in an Iranian CMTX1 family

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X-linked Charcot-Marie-Tooth (CMTX) neuropathy is the second most frequent form of CMT disease which is a genetically heterogenous group of hereditary motor and sensory neuropathies characterized by slowly progressive weakness and atrophy, primarily in the distal leg muscles. The dominant CMTX1 locus on chromosome Xq13.1 accounts for about 90% of the X-linked cases and is usually associated with mutations in the gap junction protein  $\beta 1$  (*GJB1*) gene which encodes for connexin 32 (*Cx32*), an integral transmembrane protein that involves in the transport of small molecules within Schwann cells.

Blood samples from 32 Iranian families and more than 150 members of their families with diagnosis of CMT disease, either axonal or demyelinating, were obtained for genetic analysis of *PMP22*, *MPZ* and *GJB1* genes. Total genomic DNA was extracted from all family members using standard procedures. The 1.5 Mb CMT1A duplication and point mutations both in *PMP22* and *MPZ* (CMT1B) genes were first excluded using restriction enzymes and sequencing respectively. The *GJB1* gene was then investigated with three markers (DXS7132, DXS981 and DXS6789) linked to the gene. Then PCR amplification and direct sequencing of coding exon 2 and nerve-specific P2 promoter region performed for X-linked patients. In this study we describe a case of CMTX1 in 2 members of an Iranian family caused by a novel point mutation in the *GJB1* gene (M194I). This transversion wasn't detected in normal subjects which supports the hypothesis that it is responsible for the CMTX phenotype.

#### P16.35

#### Genetic epidemiology of Charcot-Marie-Tooth in the general Norwegian population. A mutation analysis of *PMP22*, *Connexin32*, *MPZ*, SIMPLE, *MFN2* and *EGR2*

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**Background.** The frequency of the different genotypes of Charcot-Marie-Tooth (CMT) is not well described in population based samples.

**Methods.** The CMT patients were recruited from the Institute of Medical Genetics, University of Oslo and Departments of Neurology, Neurophysiology and Paediatric in eastern part of Akershus County, Norway. The probands and their families had a clinical medical genetic investigation and a neurological examination by geneticist and neurologist GJB. Families without the *PMP22* duplication had a sequential mutation analysis of the genes: *Connexin32*, *MPZ*, *PMP22*, SIMPLE, *MFN2* and *EGR2*

**Results.** We identified 259 persons with CMT in eastern Akershus County. They were from 150 families. The estimated prevalence of CMT is 1 in 1,200 in eastern Norway. The *PMP22* duplication was found in 7.3% of the families and in 14.3% of those with CMT. Point mutation of *Connexin32*, *MPZ*, *MFN2*, *EGR2*, *PMP22* and SIMPLE were found 3.3%, 3.3%, 0.7%, 0.7%, 0.7% and 0.0% of the families, and in 3.4%, 2.3%, 0.8%, 0.4%, 0.4% and 0.0% of those with CMT, respectively.

**P16.36****A novel connexin32 mutation cause X-linked Charcot-Marie-Tooth disease in Belarus family****T. Asadchuk, K. Mosse, N. Rumyantseva;****National Center of Research and Applied Medicine «Mother and Child», Minsk, Belarus.**

The CMT1X is X-linked type of Charcot-Marie-Tooth disease, an inherited demyelinating neuropathy, associated with mutations in Cx32 gene (GJB1) coding for the gap junction protein, connexin 32. The majority of GJB1 mutations are missense mutations.

We report a family with asymptomatic mother and her two affected sons from different marriage. Males have severe motor and sensory neuropathy with walking difficulties, steppage gait, prominent muscular atrophy of muscles below the knee, distal weakness, sensory loss, and decreased tendon reflexes. The symptoms are revealed in their hands as well. Both brothers have shown clinical signs by the age of 10-11 years.

At the first stage CMT1A was excluded by QF-PCR analysis of duplication in 17p11.2 region. Then direct DNA sequencing analysis of Cx32 gene revealed a novel exon 2 (part 1) mutation identified in all three samples. Mutation c.149C>G in E1 extracellular domain resulted in a serine at codon 50 being replaced by cysteine (Ser50Cys). This substitution was not detected in healthy members of the family.

For only 10% mutations in the GJB1 gene, family history of CMT1X disease is documented so far. New mutation is associated with severe phenotype and its analysis is crucial for CMT1X diagnosis and genetic counseling for probands and their relatives.

**P16.37****A novel mutation in MFN2 gene in a patient with CMT type 2A****M. T. Akbari<sup>1,2</sup>, S. Zare Karizi<sup>2</sup>, H. Nemat-Farahzadi<sup>2</sup>, G. A. Shahidi<sup>3</sup>, M. Karamipoor<sup>4</sup>;**<sup>1</sup>Department of Medical Genetics, Tarbiat Modares University, Tehran, Islamic Republic of Iran, <sup>2</sup>Tehran Medical Genetics Laboratory, Tehran, Islamic Republic of Iran, <sup>3</sup>Iran Medical Sciences University, Tehran, Islamic Republic of Iran,<sup>4</sup>Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Islamic Republic of Iran.

Charcot-Marie-tooth neuropathies (CMT) are a group of genetically heterogeneous disease of the peripheral nervous system. Ten different genes have so far been identified causing various subtypes of CMT type 2. Mutations in the mitofusin 2 (MFN2) gene have been related to the axonal type of CMT2A. CMT2A is inherited in an autosomal dominant manner. The clinical phenotype is known to be severe and symptoms develop early. MFN2 gene encodes a mitochondrial GTPase protein. Here we report a MFN2 mutation in a CMT Iranian patient with late onset and mild phenotype. These features differ from what was expected. All exons of MFN2 gene were screened by direct sequencing and a *de novo* novel missense mutation was detected. This missense mutation, c.1574 A>G (N525S), is in exon 13 and the amino acid is located in the region between the transmembrane domain and the first coiled coil region of the polypeptide chain. To date, most of the mutations reported in MFN2 gene were missense mutations and most of them were located in the GTPase domain and the region linking the GTPase domain and the first coiled coil region. The effect of this mutation and the consequent amino acid change on the stability of the mitofusion 2 protein was studied by using Mupro ([www.mupro.proteomics.ics.uci.edu](http://www.mupro.proteomics.ics.uci.edu)) online software, which predicted that this mutation decreases the structural stability by 94 % score. However, we feel further work; either functional or modeling studies are required to establish the actual effect of this mutation.

**P16.38****Mutation spectrum and genotype phenotype correlation for dynamin 2 gene in autosomal dominant centronuclear myopathy : from neonatal to adult forms.****E. Schaefer<sup>1</sup>, C. R. Pierson<sup>2</sup>, A. Toussaint<sup>3</sup>, E. Taylor DeChene<sup>2</sup>, C. Poirson<sup>3</sup>, C. Kretz<sup>3</sup>, A. Nicot<sup>3</sup>, J. Boehm<sup>3</sup>, N. Dondaine<sup>1</sup>, L. Bonne-DeWaele<sup>4</sup>, V. Drouin-Garraud<sup>5</sup>, A. Echaniz-Laguna<sup>6</sup>, C. Jern<sup>7</sup>, H. Karasoy<sup>8</sup>, A. Krause<sup>9</sup>, B. Leheup<sup>10</sup>, J. Melki<sup>11</sup>, L. Merlini<sup>12</sup>, A. Urtizberea<sup>13</sup>, C. Wallgren-Pettersson<sup>14</sup>, E. Zanotelli<sup>15</sup>, J. Mandel<sup>1,3</sup>, A. Beggs<sup>2</sup>, J. Laporte<sup>3</sup>, V. Biancalana<sup>1</sup>;**<sup>1</sup>Laboratoire Diagnostic Génétique et EA3949. Faculté de Médecine - CHRU, Strasbourg, France, <sup>2</sup>Genetics Division, Children's Hospital Boston, Harvard Medical School, Boston, MA, United States, <sup>3</sup>IGBMC, Illkirch, France, <sup>4</sup>Odense

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Centronuclear (myotubular) myopathies (CNM) are characterized by muscle weakness and abnormal centralization of nuclei in muscle fibres. The severe neonatal X-linked form is due to mutations in the MTM1 gene. Mutations in the amphiphysin2 (BIN1) gene have been identified in autosomal recessive cases with childhood onset and moderate severity.

Mutations in the Middle domain and in the Pleckstrin Homology (PH) domain of dynamin2 (DNM2) have been found in patients with autosomal dominant inheritance. Adulthood onset and mild severity are the common features in the majority of mutations. However some mutations are associated with neonatal onset. Whereas many mutations are localised in PH domain, this domain has also been found mutated in patients with dominant Charcot-Marie-Tooth neuropathy.

We sequenced the DNM2 gene in 70 families with CNM, including 13 families with dominant inheritance. We found mutations in 10 families with dominant inheritance and in 24 sporadic cases. These mutations included 21 missense in the Middle domain, 11 missense and 1 splice site deletion in the PH domain.

These results confirm that a DNM2 defect is the main cause of adulthood form of CNM, but is also a significant cause of neonatal onset CNM, as we found 9 cases with this form mutated in exons 8 or 16 (de novo). Our study, concerning 34 new families, confirms the variability in onset and severity and supports a screening strategy based on hot spot mutations and genotype phenotype correlation.

We will present the functional connections between the 3 known proteins mutated in CNM.

**P16.39****Merosin-deficient congenital muscular dystrophy (MDC1A) in Russian patients.****T. B. Tiburkova, O. A. Schagina, E. L. Dadaly, G. E. Rudenskaya, A. V. Polyakov;****Research Centre for Medical Genetics, Moscow, Russian Federation.**

MDC1A (MIM 607855) is a severe autosomal recessive disorder caused by *LAMA2* mutations. The gene encodes alpha-2 chain of laminin, or else merosin. *LAMA2* mutations produce complete or, less often, partial merosin deficiency. In European populations, MDC1A amounts to 30-50% of all congenital muscular dystrophies cases. DNA diagnostics of MDC1A is complicated by the gene large size and absence of major mutations. Though, by now, about 100 *LAMA2* mutations were registered in MDC1A patients. Immunohistochemical detection of merosin in muscles is another diagnostic possibility. Four unrelated Russian patients with MDC1A presentation were examined for *LAMA2* mutations by direct sequencing of all coding exons including exon-introns boundaries. In two patients, four mutations were detected. The genotypes were c.3829C>T/ c.7536delC, and c.5422C>T/ c.7701delTinsGTGTCCTAGGTGTCCCTA, two of the mutations are novel. The phenotypes were typical for 'classic' MDC1A, i.e. congenital muscular weakness with hypotonia, areflexia, and early contractures, high CPK level (700-1300 U/l), EMG signs of muscle lesion with concomitant hypomyelinating polyneuropathy, unspecific dystrophic pattern on muscle biopsy, and characteristic periventricular white matter abnormality on MRI. On follow-up to 4-6 years, the patients never walked and were severely disabled physically but showed no clinical signs of cerebral involvement. Two patients in whom mutations were not found may have extensive deletions undetectable by routine methods. These families need immunohistochemical MDC1A confirmation after which indirect DNA prenatal testing with STR-markers for 6q22-q23 region could be performed.

**P16.40****Post mortem Duchenne muscular dystrophy DNA diagnostics from archival milk teeth**C. Stein<sup>1</sup>, W. M. Schmidt<sup>2</sup>, D. Jovanovic<sup>2</sup>, H. Rehder<sup>3</sup>, R. E. Bittner<sup>2</sup>;<sup>1</sup>Department of Forensic Medicine, Medical University of Vienna, Vienna, Austria, <sup>2</sup>Neuromuscular Research Department, Center of Anatomy & Cell Biology, Medical University of Vienna, Vienna, Austria, <sup>3</sup>Department of Medical Genetics, Medical University of Vienna, Vienna, Austria.

The X-linked Duchenne muscular dystrophy (DMD) remains an untreatable lethal disease causing the premature death of affected patients in their early twenties. DMD is caused by mutations in the highly complex, 79 exons spanning DMD gene. Since the characterization of the complete DMD coding sequence in the late 1980ies, thousands of different mutations scattered throughout the gene have been reported. If a DMD-patient has died prior to this era, the molecular basis for his disease remained unresolved, which hampers the accurate and direct genetic counseling of his family members, such as the establishment of carrier-diagnosis in female relatives.

Here, we report on the successful post mortem analysis of the DMD gene in two different cases. In both cases, we could get hold of preserved milk teeth of the deceased patients and successfully isolated genomic DNA thereof. Subsequently, we subjected these DNA-samples isolated from milk teeth to multiplex ligation-dependent probe amplification (MLPA)-based screening for whole exon deletions or duplications within the DMD gene. In both cases we could detect the pathogenic mutation, i.e. an out-of-frame deletion of exon 21 in one case and an out-of-frame consecutive deletion of exons 46-48 in the other.

In both cases reported here, the DNA samples extracted from teeth (which had been kept for more than 20 years and more than 40 years, respectively) were suitable for MLPA and PCR procedures and were the biologic source for establishing the causative mutation within the largest gene known, the DMD gene, even decades after the patient's death.

**P16.41****Comparative clinical and molecular analysis of DMD gene deletions and duplications**E. Neagu<sup>1</sup>, G. Girbea<sup>1</sup>, A. Constantinescu<sup>1</sup>, C. Constantinescu<sup>1</sup>, D. Iancu<sup>1</sup>, G. Talpes<sup>1</sup>, E. Manole<sup>2</sup>, E. Ionica<sup>3</sup>, N. Butoianu<sup>4</sup>, D. Plesca<sup>5</sup>, A. Todoran<sup>6</sup>, L. Barbăilii<sup>1</sup>;<sup>1</sup>National Institute of Legal Medicine "Mina Minovici", Bucharest, Romania,<sup>2</sup>Neuropathology Clinic - Colentina Hospital, Bucharest, Romania, <sup>3</sup>Multiuser Research Unit – University of Bucharest, Bucharest, Romania, <sup>4</sup>Neuropediatric Clinic - "Al.Obregia" Hospital, Bucharest, Romania, <sup>5</sup>Pediatric Hospital "V. Gomoiu", Bucharest, Romania, <sup>6</sup>Medical University Targu Mures, Bucharest, Romania.

Deletions and duplications in dystrophin gene are known to cause approximately 65% of Duchenne and Becker muscular dystrophies (DMD/BMD). To detect these major DMD gene mutations multiplex ligation-dependent probe amplification (MLPA) was applied on samples from 107 unrelated patients with clinical diagnostic or clinical suspicion of DMD/BMD. In our study, by comparing the major clinical and mutational features (location, extent, predicted effect on gene reading frame) of the 11 duplications lot and the 55 deletions lot, we have tried to assess the usefulness of the molecular analysis for the prediction of the clinical form severity and natural history of DMD/BMD in order to improve their clinical management.

**P16.42****Mutation-associated exon skipping pinpoint localization of exonic splicing regulatory elements in the DMD gene**S. Le Guedard-Mereuze<sup>1</sup>, D. Thorel<sup>2</sup>, D. Méchin<sup>2</sup>, C. Saquet<sup>2</sup>, J. Miro<sup>3</sup>, P. Khau van Kien<sup>2</sup>, M. Claustres<sup>2,1,3</sup>, S. Tuffery-Giraud<sup>1,3</sup>;<sup>1</sup>Inserm U827 - Laboratoire de Génétique Moléculaire, Montpellier, France,<sup>2</sup>CHU Montpellier - Laboratoire de Génétique Moléculaire, Montpellier, France,<sup>3</sup>Université Montpellier 1 - Faculté de Médecine, Montpellier, France.

Mutation-associated exon skipping has been recognized in an increasing number of genes as a novel form of splicing mutation. Not only nonsense mutations, but also missense and even translationally silent mutations can lead to alternative splicing events. In the DMD gene, the identification of a mutation introducing a premature stop codon in patients with a milder (Becker-like) phenotype than expected (Duch-

enne-like) is a pointer of such mechanisms. The rescue of the phenotype results from the partial elimination of the truncating mutation from dystrophin mRNA by skipping of an in-frame exon. A large fraction of these mutations are supposed to exert their effects by disrupting the activity of Exonic Splicing Enhancers (ESEs), but repression of splicing may also be due to the creation of an Exonic Splicing Silencer (ESS). Using cDNA-based mutation detection analysis, we have identified 9 novel mutations in exons 19, 29, 38, 39, 59 and 66 of the DMD gene that lead to different extents of misspliced transcripts in Duchenne (DMD) and Becker (BMD) patients. To further elucidate the underlying mechanisms, disruption of cis-regulatory splicing sequences was assessed by using the Human Splicing Finder software (<http://www.umd.be/HSF>). Seven out of the 9 mutations were found to abrogate one or several ESE(s). An ESE-dependent splicing assay has been set up to confirm the *in silico* predictions. This study illustrates how disease-causing mutations can contribute to shed light on new modulatory elements important for splicing in the DMD gene, which may be important for the exon-skipping therapeutic strategy.

**P16.43****Analysis of the dystrophin gene in Duchenne muscular dystrophy patients from Bashkortostan Republic of Russia**I. Khidiyatova<sup>1</sup>, I. Gilyazova<sup>1</sup>, I. Khidiyatova<sup>2</sup>, R. Magzhanov<sup>2</sup>, E. Khusnutdinova<sup>1</sup>;<sup>1</sup>Institute of Biochemistry and Genetics, Ufa, Russian Federation, <sup>2</sup>Bashkir State Medical University, Ufa, Russian Federation.

Duchenne muscular dystrophy is caused by mutations in the *dystrophin* gene (*DMD*) consisting of 79 exons. It is known that 25%-75% of all *DMD* gene mutations in different populations are huge deletions covering one and more exons and located in 2 hot points - 5' (6-19 exons) and 3' (40-53 exons) regions. More than 6% of mutations are large duplications, the rest are point mutations.

Using the multiplex PCR we studied mutations spectrum of twenty exons (3, 4, 6, 8, 13, 17, 19, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 60) and promoter region of *DMD* gene in patients from 60 unrelated families, living in Bashkortostan Republic (the territory of the Southern Urals). Exon deletions of the gene were revealed in 31.75% of families - 30% in 5'-region, 70% - in 3'-region. SSCP-analysis and consequent sequencing of DNA samples without large deletions revealed 2 new point mutations not described before and 1 polymorphism. In 3 patients from 2 families of Russian ethnic origin we found c.401\_404delCCAA (p.Thr134ThrfsX7) in the 6<sup>th</sup> exon; its frequency is 3.17% among unrelated patients. Mutation c.662GdelA (p.Lys2210ArgfsX11) (1.6%) was revealed in 46<sup>th</sup> exon in 1 patient in Tatar ethnic origin. We revealed mutation c.7728T>C (Asn2575Asn) in the 53<sup>rd</sup> exon, the functional significant of which is unknown. Thus, specific mutation spectrum of *DMD* gene was found in patients from Bashkortostan Republic that is very important for analysis of structural and functional features of the gene and for optimal DNA-diagnostics approaches development.

**P16.44****mRNA-based analysis of point mutations in DMD gene in a selected cohort of Spanish patients**J. Juan Mateu<sup>1,2</sup>, M. Rodriguez<sup>1,2</sup>, M. Moragues<sup>3</sup>, L. Gozalez-Quereda<sup>1,2</sup>, M. J. Barcelo<sup>1,2</sup>, J. Colomer<sup>4</sup>, A. Nascimento<sup>4</sup>, E. Tizzano<sup>1,2</sup>, P. Gallano<sup>1,2</sup>;<sup>1</sup>Genetics Dept Hospital Sant Pau, Barcelona, Spain, <sup>2</sup>CIBERER, Spain,<sup>3</sup>CIBERNED, Barcelona, Spain, <sup>4</sup>Neurology Dept Hospital Sant Joan De Déu, Barcelona, Spain.

The most common form of Duchenne and Becker muscular dystrophies causing mutations are large intragenic deletions and duplications that account for 60 to 70% of all cases. The remaining cases are due to small mutations consisting in nonsense mutations, missense mutations, splicing mutations, frameshift small deletions or insertions and midintronic insertions. The detection of these small mutations in routine diagnosis have long been a difficult issue due to the complexity and large size of the DMD gene. In order to identify these mutations we have chosen a previously described method based on whole sequencing of mRNA from muscle biopsy.

To validate the method, we studied a selected cohort of nine unrelated patients with clinical and immunohistochemical data compatible with distrophinopathy and without deletions or duplications in the DMD gene. A total of new seven mutations were found, six consisting in different nonsense mutations and the remaining consisting in a acceptor

splice change that produces the skipping of exon 25. This strategy allows 1) straightforward and rapid analysis of the whole DMD gene, 2) the detection of virtually all mutations, 3) further understanding of the molecular effect of such changes.

#### P16.45

#### Mutation Analysis of Limb Girdle Muscular Dystrophy in the Czech Republic

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Limb girdle muscular dystrophy type 2A (LGMD2A) is an autosomal recessive disorder characterized by atrophy and weakness of proximal girdle muscles. LGMD2A is caused by mutations in the CAPN3 gene (15q15) that encodes the muscle specific protein, calpain-3 (p94). LGMD2A is the most frequent form of LGMD in many European countries.

Until now, more than 300 pathogenic mutations have been found in the CAPN3 gene. We performed analysis of the CAPN3 gene in LGMD2A patients at both the mRNA level using reverse transcription-PCR or at the DNA level using PCR and direct sequencing. We screened 138 unrelated patients with preliminary diagnoses of limb girdle muscular dystrophy for mutations in the CAPN3 gene. 39 patients (28%) were found to carry mutations in the CAPN3 gene. We detected 16 previously reported mutations and 3 novel mutations (c. 802\_945del, c. 1783\_1788del, p.Q619X).

Our results show that mutation 550 delA is the most frequent CAPN3 defect in Czech LGMD2A patients (53%). In total, 33 % of the patients with mutation in the CAPN3 gene are homozygous for c.550delA, and 64 % carry it at least on one allele. Other frequent mutation is 598\_612del (11%), both mutations are localised in the 4. exon.

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#### P16.46

#### CAPN3 mutations in Russian patients with limb-girdle muscular dystrophy, type 2A

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Limb-girdle muscular dystrophy, type 2A (LGMD2A, MIM 253600) is considered the most frequent autosomal recessive MD almost everywhere in the world. The causative gene is CAPN3 located in chromosomal region 15q15.1-q21.1 and encoding a muscle-specific protease, calpain 3. Common mutations are collected mostly in eight of CAPN3 24 exons, namely, exons 4, 5, 10, 11, 12, 20, 21, and 22. Forty-two unrelated Russian patients with clinical presentation of LGMD were screened for common CAPN3 mutations by direct sequencing of the eight exons. In 17 patients (40.5%), seven already known mutations were detected: c.550delA, c.598-612del, c.649G>A, c.706G>A, c.1250C>T, c.2243G>A, and c.2305C>T. In 10 patients, mutations only in one allele were identified which points to a significant proportion of infrequent CAPN3 mutations. Mutation c.550delA was found in 13 of 17 patients (76.5%) in homozygous (3 cases), compound heterozygous (3) or heterozygous (7) state, thereby in 44.4% (16/34) of affected alleles. Thus, in Russia, c.550delA is the most common CAPN3 mutation, as well as in Bulgaria, Croatia, Slovenia, and North Italy. Other six mutations were detected in single patients and not in homozygous state. Of 17 patients, 16 were of Slavic (Russian, Ukrainian, Byelorussian) and one of Korean ethnicity; only one case was familial. All patients showed typical involvement of pelvic and shoulder girdle muscles, with no cardiomyopathy, and with calf hypertrophy in some. Age of onset varied greatly (from 3 to 34 years) as well as the disease progression. Serum creatine kinase level was also highly variable, 350—9800 U/l.

#### P16.47

#### A novel mutation in the Ryanodine-Receptor Gene (RYR1) in Malignant Hyperthermia: case report

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Malignant hyperthermia (MH) is an autosomal dominant, life-threatening pharmacogenetic disorder that is one of the causes of death during anaesthesia with volatile anaesthetic agents and succinylcholine and in the early postoperative period. The disease is genetically heterogeneous, with mutations in the ryanodine receptor gene (RYR1) at 19q13.1 accounting for up to 80% of the cases. To treat developed MH Dantrolene is used.

A 57 y.o. male patient underwent thyroidectomy in 2003 in State Hospital in Riga and developed following symptoms: EtCO<sub>2</sub> max 80 mmHg, HR till 150 beats/min, T 40.8°C, CPK 1616 U/l after anaesthesia with sevoflurane, fentanyl and succinylcholine. Dantrolene was not available in Latvia and patient died in 5 hours after anaesthesia. According to EMHG guidelines *in vitro* contracture test (IVCT) and DNA analysis were performed for the MH patient's relatives.

IVCT revealed positive results with halothane and caffeine for two of MH-like patient's family members. The functional investigation of RYR1 function in *ex vivo* tissues discovered the significant statistical difference in rest Ca<sup>2+</sup> concentrations in MHs and MHn myotubes was found. The half-maximal action's concentration with 4CmC was significantly lower in comparison with control group (p= 0.002).

Novel mutation G528T in gene RYR1 was found for four family members. Mutation G528T is a missense mutation that affects the ryanodine receptor type1 protein structure.

We consider that this novel mutation the G528T should be included in MH causative RYR1 Mutations list, though the EMHG guidelines reclaim the necessity to find the same mutation in other unrelated family.

#### P16.48

#### Alteration of expression of muscle-specific isoforms of FXR1P in facio scapulohumeral muscular dystrophy patients

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The Fragile X Mental Retardation-Related 1 (FXR1) gene belongs to the Fragile X Related family, that also include the Fragile X Mental retardation (FMR1) gene involved in Fragile X syndrome, the most common form of inherited mental retardation. While the absence of FMRP impairs cognitive functions, inactivation of FXR1 has been reported to have drastic effects in mouse and xenopus myogenesis. Seven alternatively spliced FXR1 mRNA variants have been identified, three of them being muscle-specific. Interestingly, they encode FXR1P isoforms displaying selective RNA-binding properties. Since FacioScapuloHumeral muscular Dystrophy (FSHD) is an inherited myopathy characterized by altered splicing of mRNAs encoding muscle-specific proteins, we have studied the splicing pattern of FXR1 mRNA in myoblasts and myotubes of FSHD patients. We show here that FSHD myoblasts display an abnormal pattern of expression of FXR1P isoforms. Moreover, we provide evidence that this altered pattern of expression is due to a specific reduced stability of muscle-specific FXR1 mRNA variants, leading to a reduced expression of FXR1P muscle-specific isoforms. Our data suggest that the molecular basis of FSHD not only involves splicing alterations, as previously proposed, but may also involve a deregulation of mRNA stability. In addition, since FXR1P is an RNA-binding protein likely to regulate the metabolism of muscle-specific mRNAs during myogenesis, its altered expression in FSHD myoblasts may contribute to the physiopathology of this disease.

#### P16.49

#### Molecular diagnostics of DMD gene in Polish patient with Duchenne/Becker muscular dystrophy

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Duchenne/Becker muscular dystrophy is lethal, recessive, X-linked disease, characterized by progressive muscular weakness and degeneration of skeletal muscles. It is caused by mutations within the dystrophin gene. Approximately 60% of DMD and BMD patients carry large deletions comprising even several exons. The remaining mu-

tations are duplications (6%) and point mutations (34%). Deletions and duplications causing frame shift result in the more severe DMD, whereas mutations maintain the reading frame cause the milder BMD. Deletions and duplications are focused in two "hot spots". Point mutations are scattered within whole DMD gene and difficult to identification. The most efficient method of deletion and duplication screening is MLPA technique, which allows identifying 100% of this kind of mutation and upgraded routine molecular diagnostics of this disease. Additional advantage of MLPA is possibility of carrier status determination. To find a point changes we use electrophoretic screening techniques as single stranded conformers polymorphism, heteroduplex analysis and sequencing to identifying. In our studies we analyzed 150 patients with progressive muscular dystrophy from Great Poland and Silesia region. DNA from 72 patients was analyzed by MLPA and remaining 78 by PCRmultiplex. Summarising, we identify 69 cases of deletion, 14 duplication and 3 point mutation. We did not observed deletion and amplification of whole gene. Deletions and duplication included only one exon occurred in 32% of patient. In central part of a gene appeared 57% of this mutation and in proximal 43%. MLPA technique improved significantly effectiveness of molecular diagnostics of DMD/BMD and allows to identificate of carriers.

#### P16.50

##### The novel mutation in *CLCN1* gene resulting in recessive form of the myotonia congenita.

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Myotonia congenita (MC) is a hereditary muscle disorder characterized by delayed relaxation of skeletal muscle after voluntary contraction (myotonia). MC caused by mutations in the skeletal muscle chloride channel gene *CLCN1* (7q35). The phenotypic spectrum of myotonia congenita is very diverse from mild myotonia detected only by clinical examination to severe myotonia with transitory weakness (TW) and myopathy especially in recessive cases. TW correspondents with a transitory depression (TD) of the compound muscle action potential (CMAP) during repetitive nerve stimulation (RNS). We analyzed 35 families with revealed myotonia by clinical examination in dominant or recessive forms and detected 11 mutations in 14 families. Two of these mutations detected in several unrelated families: c.1436\_1449del in exon 13 of *CLCN1* gene and p.493Ala>Glu. The last mutation is more interest. This novel missense mutation is detected in compound with c.1436\_1449del in one patient and in homozygote in two out of three sibs in another family. Clinical symptoms of affected sibs are moderate myotonia of masseter, transitory weakness in hands and "warm-up" effect, hypertrophy of calf, no exacerbation with decrease of temperature, the myotonia of lid is absent, taking of alcohol provoke the alleviation of symptoms. The myotonia don't progress in time (now sibs are 36 and 40 years old). At the same time this mild form of myotonia caused by mutation 493Ala>Glu in homozygote combines with high level of decrement CMAP RNS (70 and 78%). By now, routine analysis of *CLCN1* gene for patients with myotonia is going on.

#### P16.51

##### Molecular diagnosis of myotonic dystrophy type I in Egyptian patients

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The dominantly inherited myotonic dystrophy or myotonia dystrophica (DM) disease belongs to a group of neurodegenerative disorders that results from the expansion of unstable trinucleotide repeats. In these disorders, the number of repeats increases to a critical size, affecting the gene function and leading to disease. The DM disorder has a peculiar and rare pattern of multisystemic clinical features, affecting skeletal muscles, heart, eye and endocrine system. The underlying molecular basis of the disease entails excessive expansion of a repetitive sequence in the myotonin protein Kinase gene.

The aim of this study was to employ a molecular diagnostic technique for the disease among suspected Egyptian cases.

Subjects and Methods: Nine probands complaining of clinical and neurophysiological features suggestive of myotonic dystrophy diagnosis were studied. Molecular studies were performed on the nine probands

and their available family members. Genomic leukocytic DNA samples were extracted using a salting out technique. A polymerase chain reaction primer pair was used to amplify the designated fragment followed by estimation of the CTG repeat length. A modified nucleotide mixture was used for amplification of the expanded premutated and fully mutated CTG alleles.

Results and discussion: Five families out of the nine showed abnormal repeat expansion. The PCR products were directly detected on 8% ethidium bromide stained-polyacrylamide gel. The utilization of molecular methods in DM management would not only lead to definitive molecular diagnosis and proper genetic counseling for affected families but may also aid in future prenatal diagnosis.

#### P16.52

##### GTG repeat polymorphism in myotonic dystrophy patients

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Myotonic dystrophy (DM) is a neuromuscular disorder with autosomal dominant inheritance pattern. It affects various organs including skeletal muscle, heart, brain, eye, endocrine, and gastrointestinal systems. It is characterized with progressive muscle weakness and wasting and difficulty in muscles relaxation after contraction (myotonia). It is caused by an excessive number of CTG repeats at chromosome 19q13.2-13.3 region.

It has four subtypes. These are classified as; premutation, mild, classical and congenital. These subtypes are named according to changes in repeat numbers. In a normal person CTG repeat number can go up to 35. Trinucleotide repeat number between 35 and 50 is not attributed to a disease condition though they are not stable and are inherited from one generation to the next.

Low trinucleotide repeat numbers can be readily amplified with PCR. High repeat numbers, however, are hard to amplify. In this study in order to amplify alleles with high CTG trinucleotide repeat numbers, "Triple PCR" was used.

Study group was consisted of 13 unrelated DM1 patients admitted to the Department of Neurology in Çukurova University School of Medicine. After the proband was diagnosed as DM1, other family members were assessed and the diagnosis was confirmed genetically. By the data obtained from the probands, the pedigrees were constructed. Thirty nine members including 20 men and 19 women of 13 unrelated DM families were analyzed for CTG trinucleotide repeat polymorphism to diagnose the DM1 patients on a molecular basis.

Method of Triple PCR had positive results in all DM1 patients and proven to be a reliable diagnostic tool.

#### P16.53

##### Paternal repeat length instability of myotonic dystrophy type 1 pre- and protomutations

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Myotonic dystrophy type 1 (DM1) is an autosomal dominant disorder, and the most common form of muscular dystrophy in adults. The molecular basis of DM1 lies in the instability of a CTG repeat in the 3' UTR of the DM1 protein kinase gene. Small expanded DM1 alleles are usually unstable and often increase in number in successive generations. Here we evaluated the risk on expansion of an additional 22 pre- (37-50 CTG repeats) and 41 protomutation (50-80 repeats) in relation to sex and repeat length in DM1 transmitting parents for 63 DM1 parent-child pairs (33 males, 30 females). CTG-repeat lengths in the parents and children were determined by PCR and Genescan analysis. For the transmitting males, 23/33 (70%) repeats expanded to a full mutation upon transmission, whereas 5/33 (15%) repeats were stable and 5/33 (15%) slightly increased. For the transmitting females, these values were 5/30 (17%), 17/30 (57%) and 6/30 (20%), respectively. When the results were subdivided by repeat length, only one (1/8, 13%) transmitting male gave rise to a full mutation in the offspring in the premutation range, and 9/11 (82%), 6/6 (100%) and 7/8 (86%)

in the protomutation ranges, 51-60, 61-70 and 71-80 repeats, respectively. For the transmitting females, full mutations were only seen in the 71-80 repeat range, and in 5/6 (83%) offspring.

In conclusion, paternal repeat length instability occurs frequently and mainly between 51-80 repeats, while maternal repeat length instability occurs infrequently and only above 70 repeats. This observation has implications for genetic counseling.

#### P16.54

##### Unusual splice site mutation involved in a fatal form of TK2 associated mitochondrial DNA depletion myopathy

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Mutations in the TK2 gene are associated with the autosomal recessive mtDNA depletion myopathy. Here, we describe two dizygotic twins who developed progressive, severe muscle hypotonia by age of 10 months. Both had elevated serum CK (>1000 U/L) and MRI disclosed marked cortical atrophy. Muscle biopsy revealed red-ragged fibres, multiple sarcoplasmic vacuoles, and numerous cytochrome-c-oxidase negative fibres. Activities of respiratory chain enzymes were massively reduced. After detection of pronounced mtDNA copy number reduction in muscle, we sequenced TK2.

We found compound heterozygosity for two mutations in both siblings: c.542C>T (p.A181V) and c.665-7A>G (IVS7). The mother was heterozygous for c.542C>T, and the father carried the heterozygous c.665-7A>G mutation. Whereas pathogenicity of c.542C>T had been previously established, the second mutation has not been reported so far. Several in silico predictions for this mutation, which affects position -7 of the IVS7 acceptor splice site, failed to suggest any detrimental effect on splicing. To investigate the potential pathogenicity of this intronic base substitution, RNA isolated from peripheral blood was subjected to RT-PCR analysis, revealing that the mutation creates an aberrant acceptor splice site leading to inclusion of extra nucleotides (r.664\_665insCCTCAG). This aberrant splicing pattern ultimately causes the predictable intolerable insertion of two additional amino acids [p.(221\_222insAlaSer)] into a highly conserved motif within the deoxyribonucleoside kinase domain.

Our work not only extends the TK2 mutational spectrum, it also highlights the importance of experimental analysis of intronic mutations detected during routine DNA diagnostics procedures, because algorithms to predict a splicing defect were uninformative in our case.

#### P17. Genetic analysis, linkage and association

##### P17.01

##### Common variants near MC4R are associated with general and visceral adiposity in European- and African-American Youth

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Objectives: Rare functional mutations of melanocortin 4 receptor (MC4R) are the leading cause of monogenic severe childhood-onset obesity. Recent genome-wide association studies (GWAS) found common variants near MC4R associated with obesity and insulin resistance (IR). This study aimed to assess the influence of the identified single nucleotide polymorphisms (SNPs) rs17782313 and rs17700633 on general and visceral adiposity, and on IR in European-American (EA) and African-American (AA) youth.

Methods: In 1902 youth (48.9% European-American, 45.4% male, mean age 16.2 years), we examined the associations of the rs17782313 and rs17700633 with anthropometry, percent body fat (%BF) and visceral adipose tissue (VAT). Interactions of the SNPs with ethnicity or gender were investigated and haplotype analyses conducted.

Results: Significant associations were found between rs17782313 and body mass index (BMI) ( $P=0.004$ ), weight ( $P=0.002$ ), waist circumference ( $P=0.004$ ), and sum of skinfolds ( $P=0.032$ ), explaining between 0.30% and 1.0% of variance. Rs17700633 was significantly associated

with %BF ( $P=0.018$ ) and VAT ( $P=0.006$ ), explaining 0.57% and 1.67% of the variance, respectively. No significant interactions of the variants with ethnicity or gender were found for any of the obesity-related phenotypes. Compared to the most common haplotype, the haplotype with both minor alleles showed higher weight ( $P=0.002$ ), BMI ( $P=0.02$ ), %BF ( $P=0.004$ ) and VAT ( $P=0.02$ ). No significant effects were found on indices of IR after adjustment for BMI.

Conclusions: The relatively large effect of these common variants near MC4R with general and visceral adiposity in childhood could prove helpful in elucidating the molecular mechanisms underlying the development of obesity in early life.

##### P17.02

##### The frequencies of polymorphic alleles +49A/G CTLA4, -1858C/T PTPN22 and -23HphiA/T INS genes in Belarusian population and diabetic patients.

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Various genes were proved to control predisposition to autoimmune diabetes (T1D). Our aim was to study the genotype frequencies of +49A/G CTLA4; 1858C/T PTPN22 and -23HphiA/T INS and their combinations in healthy Belarusians and diabetic patients. 474 native Belarusians from 6 ethnogeographic regions; 20 patients with comorbidity of autoimmune thyroid disease (AITD) and type 1 diabetes, as well as 23 type 1 diabetic patients were genotyped. The risk genotype GG-TT-AA (CTLA4-PTPN22-INS respectively) was revealed in 6 individuals (1,27 %) of ethnic population. 14 individuals (2,95 %) possessed protective homozygous genotype (AA-CC-TT) and 35 individuals (7,4 %) were heterozygotes at all loci. The comparative study of CTLA4-PTPN22 genotype frequencies was conducted in 741 individuals from 6 ethnogeographic regions. The most common genotype GA-CC (heterozygous of +49A/G CTLA4 locus and homozygous of 1858C/T PTPN22 locus) was revealed in 33,9 % of population studied. The protective genotype (AA-CC) was detected in 22,5 % of investigated Belarusians. The risk genotype (GG-TT) frequency was low - 0,9%. In diabetic subjects the risk genotype GG-TT-AA (CTLA4-PTPN22-INS respectively) was revealed in 1 case (2,3%). The GA-CT-AA genotype frequency was significantly ( $P<0,05$ ) higher in patients (25,6 %) in comparison with population (7,8%). Note that frequency of risk alleles ("T" - PTPN22 gene and "A" - INS gene) in the patients were also significantly higher (31,4 % and 89,5% vs. control subjects 16,4% and 71,3% respectively). Thus we proved the role of PTPN22 and insulin genes and their risk alleles combinations in Belarus patient cohort comparing with general population.

##### P17.03

##### A polymorphism within the fructosamine-3-kinase gene associates with HbA<sub>1c</sub> and the onset of type 2 diabetes

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Background: Non-enzymatic glycation is a process, which leads to the formation of advanced glycation endproducts. These compounds are involved in the development of late diabetic microvascular complications. Fructosamine-3-kinase (FN3K) is an intracellular enzyme that phosphorylates fructosamines resulting in fructosamine-3-phosphate, which subsequently decomposes to inorganic phosphate, 3-deoxyglucosone and the unmodified amino residue. Recently, the C900G (rs1056534) single nucleotide polymorphism (SNP) of the FN3K gene was found to be associated with the enzyme activity. The aim of the study was to investigate the impact of the SNP on clinical and biochemical features and late complications of type 2 diabetes.

Methods: Total of 859 type 2 diabetic subjects were enrolled in the study and were genotyped with PCR-RFLP method.

Results: Genotype frequencies were as follows, GG: 41%, GC: 54%, CC: 5%. Subjects with CC variant had higher HbA<sub>1c</sub> levels compared with the others (CC: 6.48±0.15%; GC: 7.66±0.09%; GG: 7.68±0.09%;  $p<0.001$ ). Furthermore, in case of the CC allelic variant type 2 diabetes was diagnosed at a later age than in case of GC or GG variants (CC:

56.0±1.90 years; GC: 52.0±0.62 years; GG: 50.1±0.71 years;  $p<0.05$ ). Logistic regression analysis did not reveal association between CC genotype and late diabetic complications, such as diabetic nephropathy, neuropathy and retinopathy (OR=0.796, CI 95% 0.364-1.744,  $p=0.569$ ; OR=1.754, CI 95% 0.806-3.393  $p=0.170$ ; OR=1.213, CI 95% 0.470-3.132,  $p=0.690$ , respectively). Conclusion: We conclude that the C900G polymorphism associates with the level of HbA<sub>1c</sub> and the onset of the disease, but not with either of the late diabetic microvascular complications.

#### P17.04

#### Heritabilities of blood pressure, fasting glucose and triglycerides depend on body mass index: evidence for gene-obesity interaction

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Background: Evidence from candidate gene studies suggests that obesity may modify the genetic susceptibility to hypertension, type 2 diabetes and dyslipidemia. Gene-obesity interactions are expected to result in different heritability estimates at different obesity levels.

Method: The present study included 1260 monozygotic and 842 dizygotic twins (mean±SD age: 37.90±9.85; range: 19.14-81.36) from the Chinese Twins Registry. Outcome measures were systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting glucose and triglycerides. Structural equation modeling was used to test whether body mass index (BMI) interacted with latent genetic and environmental effects on the outcome measures.

Results: Genetic influences on triglycerides increased with BMI ( $p<0.001$ ), which resulted in a higher heritability estimate at higher BMI levels. However, the environmental influence on SBP, DBP and fasting glucose increased with BMI ( $p<0.001$ ), which resulted in lower heritability estimates at higher BMI levels. This was confirmed by stratified analysis in twin pairs concordant for normal weight (BMI<25kg/m<sup>2</sup>) and twin pairs concordant for overweight (BMI≥25kg/m<sup>2</sup>). Heritabilities were 12 percentage points higher for triglycerides, whereas 29, 7 and 6 percentage points lower for SBP, DBP and fasting glucose, respectively, among twins concordant for overweight relative to twins concordant for normal weight.

Conclusion: Our results suggest that the expression of genes influencing triglyceride levels can vary as a function of obesity status. The substantial increase in the genetic contribution to the total variance in triglyceride and decreases in SBP, DBP and fasting glucose may prove valuable in aiding gene finding efforts.

#### P17.05

#### Association of genetic variation in KCNQ1 with type 2 diabetes in KORA

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Recently, a significant association between variants of the KCNQ1 gene and type 2 diabetes has been reported. Replication of polygenic disease associations has often revealed false positive findings and overestimated effects in initial reports in the past. Therefore, this study aimed to replicate the association of the variant rs2237895, which was originally found in an Asian population and assesses its effect on type 2 diabetes in a case-control study of 2.697 KORA participants (1230 cases/ 1467 controls). We further extended our analyses to the glycemic traits in a population based sub sample of 1092 fasting KORA participants. Results of our analyses confirmed the minor C allele as risk variant for type 2 diabetes (OR=1.12 [1.00,1.25];  $p=0.049$ ). This association could be ascribed to the association in men (OR=1.16 [1.00,1.35];  $p=0.040$ ). Assessing effects for metabolic parameters, significantly increased fasting glucose levels were found for the C allele of rs2237895 (beta estimate=1.39 mg/dl;  $p=0.01$ ) in men, supporting affection of pancreatic beta cell function by variants in KCNQ1 leading

to type 2 diabetes by means of impaired insulin secretion. However, mechanisms remain unclear and absence of the effect in women has to be elucidated in further studies. Finally, our results underline a role of KCNQ1 variants in type 2 diabetes and related traits in Caucasians.

#### P17.06

#### PIK3R1, SLC2A4 and PTPN1 expression level analysis and its association with insulin resistance development in type 2 diabetes patients

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Background: After insulin binds to its receptor, further reactions transmit the signal downstream the cell that results in glucose uptake. When the pathway is impaired, insulin resistance appeared and finally type 2 diabetes develops.

Methods: The SNP polymorphisms located in regulatory region of selected genes implicated in insulin signal transduction as well as the expression level of these genes were analyzed. The study was performed in type 2 diabetes patients with and without insulin resistance (assessed by HOMA-IR) and in healthy subjects. Expression rate and SNPs were tested in genes: PIK3R1 (rs3756668, rs1862162), PTPN1 (rs16989673) and SLC2A4 (rs5417, rs5418). SNPs were analyzed in minisequencing reaction followed by capillary electrophoresis. Gene expression analyses were carrying out as relative quantification with  $\beta$ -actin as housekeeping gene.

Results: PIK3R1 and PTPN1 exhibited correlation between HOMA-IR and expression level ( $R=-0.5485$  and  $R=-0.5257$ , respectively). Gene expression level compared between tested groups revealed significant difference in PIK3R1 expression rate ( $p=0.0172$ ), furthermore we were close to achieve statistical importance for G/G carriers of rs3756668 and expression level of PIK3R1 ( $p=0.06$ ). The results showed no substantially difference in genes expression of PTPN1 and SLC2A4 between groups ( $p=0.7951$  and  $p=0.5547$  respectively), however we revealed association for rs5417 and rs5418 heterozygotes with lower SLC2A4 expression rate ( $p=0.0235$ ).

Conclusion: All presented data suggest that expression of genes implicated in insulin signal transduction is impaired in type 2 diabetes patients and might be associated with insulin resistance development. Furthermore, some SNP polymorphisms in regulatory regions might influence gene expression rate.

#### P17.07

#### The vitamin E binding protein Afamin is associated with the Metabolic Syndrome and underlies hormonal control

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The metabolic syndrome is characterised by metabolic risk factors including abdominal obesity, atherogenic dyslipidemia, elevated blood pressure or insulin resistance. Patients with the metabolic syndrome are at increased risk of coronary heart disease, other atherosclerotic conditions and type 2 diabetes.

We have previously observed in the population-based Bruneck study (n=826) an association of the human vitamin E binding protein afamin with several key parameters of the metabolic syndrome: by measuring its plasma concentration we found significant associations with waist-to-hip ratio, body mass index, obesity, systolic and diastolic blood pressure, diabetes, and plasma concentrations of LDL- and HDL-cholesterol, triglycerides, free fatty acids, glucose and HbA1c. In addition, afamin concentrations were also positively correlated with increasing numbers of these parameters in a 10-years follow-up prospective observation suggesting also a predictive potential of plasma concentrations of afamin for developing the metabolic syndrome.

The present study was undertaken to investigate possible influences on afamin expression. In mutant mice with a defective androgen receptor (Tfm mice) we found extremely low afamin concentrations in plas-

ma, suggesting an important hormonal regulation of afamin expression (at least in mice). We therefore performed hormone substitution experiments in Tfm and wild-type mice (by means of subcutaneously applied hormone-saturated silicone implants). Estradiol had no effect on afamin concentrations in either mice. Testosterone, in contrast, led to diminished plasma concentrations in wildtype mice and normalized the low afamin values in Tfm mice. These data suggest androgen-receptor- dependent and -independent influences of testosterone on the expression of afamin.

#### P17.08

#### Association of mitochondrial DNA 16189 T/C polymorphism in Turkish patients with Metabolic Syndrome

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Metabolic syndrome (MetS) is a cluster of several clinical conditions including dyslipidemia, hypertension, and hyperglycemia. A common variant in mitochondrial DNA (mtDNA) at 16189 bp (T/C transition) has been suggested to be related to thinness, impaired glucose tolerance/ type 2 diabetes mellitus (DM) and insulin resistance. The objective of our study is to evaluate association between the 16189 variant of mtDNA in Turkish patients with MetS. Seventy subjects with MetS and 150 healthy controls were enrolled in the study. mtDNA was extracted from peripheral leukocytes and the presence of the 16189 variant of mtDNA was determined using the PCR-RFLP analysis. The statistical difference in the frequency of occurrence of the 16189 variant of mtDNA between patients and healthy controls and its association to type II DM was assessed by the Pearson's chi-square test. Patient variables were compared between mtDNA 16189T and C carriers by Student's *t* test. P<0.01 was considered statistically significant. We found that 26% (18/70) of patients and 16% (24/150) of healthy subjects had polymorphic C variant and the difference was not statistically significant. Also, mtDNA 16189C variant did not associated with variables including BMI, HDL, total cholesterol, cortisol, triglyceride, fasting insulin, fat percentage, insulin resistance and presence of type II DM. On the other hand, patients with 16189C variant had lower LDL value (p=0.006). As a conclusion, mtDNA 16189 variant is not associated with metabolic syndrome in Turkish patients. Larger population study is necessary to clarify the effect of this variant on LDL level of the patients.

#### P17.09

#### Mutation analysis of GCK, HNF1A and HNF4A genes in Italian MODY patients

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Maturity onset-diabetes of the young (MODY) is a genetically heterogeneous group of disorders characterized by early onset non-insulin diabetes mellitus, autosomal dominant inheritance and primary defect in pancreatic beta cell function. Six genes have been associated with different subtypes of disease. The most common forms are MODY2 and MODY3, while MODY1, 4, 5 and 6 are rare disorders. Aim of our study is to assess the relative prevalence of mutations in GCK/MODY2, HNF1A/MODY3 and HNF4A/MODY1 genes among Italian patients. 102 unrelated probands fitting MODY clinical criteria were screened first for GCK mutations, when negative, for HNF1A and when still negative, for HNF4A mutations. The analysis was performed by DHPLC and direct sequencing. Mutations in GCK gene were detected in 51 (50%) families: 14 mutations were previously unreported. Mutations in HNF1A gene were detected in 5 (4.9%) probands: two were new and one was a *de novo* mutation. Only 2 (1.9%) patients showed defects in HNF4A gene. Noteworthy, one of these latter cases carried also a mutation in HNF1A/MODY3 gene. This is the first report of a patient with mutations in two MODY genes. For the novel missense and splicing mutations bioinformatics analyses were performed to predict the pathogenic effect. Unlike the studies on north European MODY patients, our study indicates that defects in GCK/MODY2 gene are a

very common cause of MODY in Italian population, whereas HNF1A/MODY3 and HNF4A/MODY1 have a low prevalence. Our data broadens the knowledge of the naturally occurring mutations repertoire in these genes.

#### P17.10

#### Non insulin dependent diabetes mellitus and insulin receptor gene mutations

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Non insulin dependent diabetes mellitus (NIDDM) is a metabolic disorder that is characterized by constant hyper glycemia and insulin resistance. Insulin receptor consists of two glycoprotein subunit, alpha and beta subunit. Beta subunit contains tyrosine kinase domain. Insulin receptor gene has twenty two exons. The mutations of insulin receptor gene were found to be associated with development of NIDDM. In this background we have investigated whether mutations of tyrosine kinase domain of insulin receptor gene is associated with type II diabetes mellitus in Iranian population. Exons fourteen to seventeen of insulin receptor gene related to tyrosine kinase domain. DNA of peripheral blood of 128 cases was extracted and four related exons to tyrosine kinase domain separately were amplified by specific primers and then PCR products were sequenced. The different mutations of insulin receptor gene in tyrosine kinase domain were detected in Iranian NIDDM patients. We reported for first time a polymorphism (C 2706 G) and two missense mutations (C 2752 T) and (C 2753 G) in exon fourteen and so a missense mutation (T 3257 A) in exon seventeen of insulin receptor gene in Iranian NIDDM patients. These mutations in Iranian population are different from previously reported mutations in insulin receptor gene of the other populations. There is not detected any mutation or polymorphism in other exons of tyrosine kinase gene.

#### P17.11

#### Apolipoprotein A1/C3/A5 gene cluster variants and serum lipids levels in patients with type 2 diabetes mellitus

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OBJECTIVE: To evaluate if there is any association between apoA1/C3/A5 gene cluster polymorphisms and serum lipids levels in type 2 diabetic patients. PATIENTS AND METHODS: 225 patients with type 2 diabetes mellitus (161 females and 64 males, mean age 57±0.4 years) not taking lipid-lowering drugs. Serum lipids were evaluated in all patients by enzymatic method. G-75A and C83T apoA1, Sst1 apoC3, S19W and -1131TC apoA5 gene polymorphisms were identified by PCR-RFLP method. RESULTS: Allele frequencies for all investigated polymorphisms in type 2 diabetic patients were the same as in Caucasian population. Fasting serum TG and VLDL-cholesterol levels were significantly higher in female patients carrying the minor allele 19W than in patients with SS genotype (3.1±0.5 mmol/L vs. 2.1±0.1 mmol/L for TG and 1.4±0.2 mmol/L vs. 1.0±0.1 mmol/L for VLDL-cholesterol, p=0.033). Total cholesterol, LDL-cholesterol, and HDL-cholesterol levels were not significantly different between genotypes. No differences were found between S19W apoA5 genotypes in the male patients. Significantly higher levels of fasting serum TG and VLDL-cholesterol were also found in female patients with GA and AA genotypes of G-75A apoA1 polymorphism compared to GG genotype patients (2.8±0.3 mmol/L vs. 2.0±0.1 mmol/L for TG and 1.3±0.1 mmol/L vs. 0.9±0.1 mmol/L for VLDL-cholesterol, p=0.009). No differences were found in serum lipids levels between C83T apoA1, -1131TC apoA5 and Sst1 apoC3 genotypes in male and female type 2 diabetic patients. CONCLUSION: We detected an association between apoA1/C3/A5 gene cluster variants and serum lipids levels in female patients with type 2 diabetes mellitus.

**P17.12****Analysis of the combined discriminative value of 11 validated obesity genetic variants**

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**Background:** Within the last years, GWA studies have identified 20 genetic variants associating with obesity or related phenotypes. The variants are common and exhibit moderate effect sizes in the general population. The aim of this study is to investigate the combined effect of these variants and their ability to discriminate between normal weight and overweight/obese individuals in a cross-sectional population of Danes, by applying receiver operating characteristics (ROC).

**Methods:** 11 variants were genotyped in 3 study groups: the population-based Inter99 study, the ADDITION Denmark screening study cohort, and a type 2 diabetic patient group sampled at Steno Diabetes Center. The combined study population included 5,512 normal weight, 7,458 overweight and 5,044 obese individuals. For the remaining 9 variants genotyping is still ongoing.

**Results:** Individual variant analyses demonstrated per allele odds ratios for the 11 variants ranging from 1.04(0.98-1.08) to 1.21(1.14-1.27). When combining the variants, individuals with extreme risk profile ( $\geq 15$  risk alleles) showed a significant increase in risk of both overweight 1.61(1.42-1.82),  $p=4.1\times 10^{-14}$  and obesity 1.68 (1.49-1.89),  $p=2.2\times 10^{-16}$ , compared to individuals with minimal risk profile (<9 risk alleles). The area under ROC curve predicting overweight and obesity was determined to 0.55 and 0.54, respectively.

**Conclusion:** The 11 variants analysed out of the 20 variants confers a significantly increased combined risk of both overweight and obesity in carriers of many risk alleles. The discriminative value of the 11 variants is still sparse and too inaccurate for clinical preventive purposes, but is expected to increase when the remaining 9 variants are included.

**P17.13****ACE polymorphisms in children with arterial hypertension**

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Arterial hypertension (AH), either essential or secondary, is an important issue in childhood for its short- and long-term cardiovascular morbidity. The frequency of arterial hypertension in children was estimated at the level of 1-5% of the population. Renal diseases are the most frequent causes of AH in children, but essential hypertension can also be detected early in life. Among the many potential causes of secondary hypertension are renal parenchymal disease, occlusive renal arterial disease, adrenocortical abnormalities, and pheochromocytoma. It is important for blood pressure to be checked regularly (at least once every year) in healthy children and adolescents and at every medical visit in those belonging to at-risk categories (family history of AH, low birth weight, obesity, etc). Essential hypertension is a multifactorial disease in which both genetic and environmental factors play important roles. The present study examines how polymorphisms of the insertion/deletion (I/D) angiotensin-converting enzyme (ACE) genes influence presence and severity of hypertension. The I/D polymorphisms of the ACE genes were determined by RFLP (restriction fragment length polymorphism) and restriction analysis in all hypertensive patients admitted in Pediatric Clinics from Tg. Mures, Romania. Genotyping was done in children diagnosed with primary and secondary AH. Genomic DNA was extracted from whole blood samples using standard methods. PCR-RFLP was used for analysis. Detection was performed with agarose gel electrophoresis. First data of this study will be presented. Acknowledgements: The study was realized in the research program

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**P17.14****The  $\beta_3$ -adrenergic receptor gene polymorphism, systolic blood pressure and body mass index in children with systolic arterial hypertension.**

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**Background:** The  $\beta_3$ -adrenergic receptor (ADRB3) gene polymorphism was reported to be associated with obesity and arterial hypertension (AH) in adults, while there are a few reports on children.

**Aim:** To investigate distribution of ADRB3 genotypes and allele frequencies of Trp64Arg polymorphism in children with systolic AH, and study clinical systolic blood pressure (SBP) levels and body mass index (BMI) values in carriers of the different genotypes.

**Methods:** 84 children, aged 7-17, with the diagnosis of systolic AH participated in this study. AH was defined as systolic/diastolic blood pressure measurements higher than 95 age-gender-height percentile of the adopted reference values. The Trp64Arg polymorphism of the ADRB3 gene was detected by PCR, the amplified PCR products were digested with Bst 2UI.

**Results:** There were identified 78.6%(66) Trp/Trp homozygotes, 19.0% (16) Trp/Arg heterozygotes and 2.4% (2) Arg/Arg homozygotes. The frequencies of Trp and Arg alleles were, respectively, 0.9 and 0.1. Clinical SBP in patients with Trp/Trp genotype was  $133.5\pm 9.1$  mm Hg, with Trp/Arg genotype was  $135.5\pm 7.9$  mm Hg, with Arg/Arg genotype was  $132.5\pm 2.1$  mm Hg. Clinical SBP difference among carriers of the different genotypes was not found. No significant difference was observed in BMI among subjects carrying the of Trp/Arg, Arg/Arg and Trp/Trp genotypes. However Arg carries with BMI < 25 percentile were not found.

**Conclusion:** The significant differences in SBP levels and IMT values among carriers of different genotypes of Trp64Arg polymorphism of ADRB3 gene were not found in children with systolic arterial hypertension.

**P17.15****A study of the Insertion-Deletion polymorphism of the gene angiotensin-converting enzyme, ACE**

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**Introduction:** We studies gene, encoding angiotensin-converting enzyme ACE is located on the chromosome 17 (17q23) consist of 26 exons and 25 introns. In given gene exists polymorphism, given by insertion 287 base pares in 16 intron region (the allele: \*D - 192 bp, \*I - 490 bp.). The ferment, coded gene, is the important physiological regulator of arterial pressure and water-salt exchange. The various degree is connected to the given polymorphism of expression the gene ACE. At persons, homozygous on allele D, the contents of enzyme grows.

**Materials And Methods:** We studied samples DNA of 319 individuals in the age of 18-35 years living in Republic Bashkortostan. Sample was divided into groups, depending on factor of intellectual development (IQ). Definition of a level of intellectual development is realized by the test of R.Kettell. The analysis genetic polymorphism is realized by polymerase chain reaction (PCR).

**Results:** We carried out the analysis of distribution of frequencies of genotypes and alleles polymorphic marker I/D of gene ACE. It is established, that in group with an attribute "endowments" increase of frequency of genotype ACE\*D/\*D (OR=2.223; 95%CI 1.219-3.098) and allele\*D (OR=2.641; 95%CI 1.219-7.074); allele\*I meets in the given group authentically less often (OR=0.379; 95%CI 0.142-0.821).

Thus, the gene ACE is associated with a level of intellectual development of the person.

## P17.16

## Genetic markers of essential hypertension, located on chromosome 1q

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Elevated blood pressure is a complex trait regulated by multiple factors. Genetic predisposition, along with lifestyle changes, plays the crucial role in the development of essential hypertension (EH). Twin studies have demonstrated that almost 50 percent of the inter-individual variability in blood pressure level is heritable. Genome-wide linkage studies have associated the incidence of hypertension with some genomic regions (chromosome 1q, 2p, 2q, 3p, 6q, 16q, 15q, 18q, 19p).

We performed screening of genetic markers located on chromosome 1q. DNA samples, used in the study, were obtained from 1095 individuals (355 Tatars, 362 Bashkirs and 378 Russians residing in Bashkortostan, Russia). SNP-genotyping of 1q candidate loci was performed using polymerase chain reaction followed by restriction enzyme digestion. Data were analyzed using Arlequin 2.0.

We found that haplotypes of polymorphic variants in E-selectine (*SELE*, rs2076059), P-selectine (*SELP*, rs6131), L-selectine (*SELL*, rs3177980), beta 1 polypeptide of Na+/K+ transporting ATPase (*AT-P1B1*, rs12731646) and regulator of G-protein signalling 5 (*RGS5*, rs2255642) genes are associated with essential hypertension. *TSLCG* haplotype was associated with increased risk of EH in ethnic Russians (OR=2.88, CI<sub>OR</sub> 1.45-5.72, P=0.002), while *TALCA* (OR=0.01, CI<sub>OR</sub> 0.01-0.82, P=0.042), *TSFCA* (OR=0.34, CI<sub>OR</sub> 0.12-0.93, P=0.033) and *TSFCG* (OR=0.44, CI<sub>OR</sub> 0.21-0.90, P=0.022) haplotypes were found to be protective against EH. Increased risk of EH in Tatar ethnic group was associated with *CAFTA* haplotype (OR=29.64, CI<sub>OR</sub> 3.92-224.30, P=0.000), decreased - with *TSFTA* (OR=0.04, CI<sub>OR</sub> 0.01-0.31, P=0.001) and *TSLCA* (OR=0.06, CI<sub>OR</sub> 0.01-0.49, P=0.033) haplotypes. Our data confirm the association between genetic markers on chromosome 1q and human hypertension.

## P17.17

Haplotypic effect of three functional promoter polymorphisms of *MMP1* confers higher risk of myocardial infarction

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## Purpose

Inherited and acquired risk factors contribute to the development of the atherosclerotic lesion and its most serious clinical manifestation, myocardial infarction (MI). Studies with human tissues and animal models have suggested a role for matrix metalloproteases (MMPs) in atherosclerosis, and several functional polymorphisms in the *MMP-1* gene have been linked to the risk for MI. The aim of this study was to evaluate the association between three promoter polymorphisms and early MI in a Spanish cohort.

## Methods

We performed a case-control study with 261 unrelated male patients who had suffered an early MI and 194 healthy matched controls. All participants were smokers and younger than 60 years. The genotypes for the three *MMP-1* promoter polymorphisms (-1607 1G/2G; -519 A/G; -340 T/C) were determined through restriction enzyme digestion of a PCR fragment (PCR-RFLP). Comparison of allele and genotype frequencies of individual polymorphisms and haplotypes was carried out using the chi-square test (SHEsis software).

## Results

Allelic and genotypic frequencies of individual polymorphisms did not differ between patients and controls. Statistical analysis of the haplotypes showed that the combination -1607<sub>2G</sub>/-519<sub>G</sub>/-340<sub>T</sub>, present in 3.4 % of controls, had a higher frequency among patients (p=0.005; OR=2.4; CI=[1.27-4.55]). Moreover, the haplotype -1607<sub>1G</sub>/-519<sub>G</sub>/-340<sub>T</sub> showed higher frequency in controls (p=0.02; OR=0.68; 95 % CI=[0.49-0.94]). This haplotype is the combination of alleles with a described less transcriptional activity.

## Conclusion

Our results confirmed and extended the previously reported association between *MMP-1* promoter polymorphisms and MI, proving that its action could be through the instability and rupture of the plaque.

## P17.18

## Relationship of the APOE polymorphism and lipid profile: a population-based study in the Azores Islands (Portugal)

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The factors leading to a two-fold mortality rate from coronary artery disease (CAD) in the Azores, as compared to Mainland Portugal, have not been elucidated. Previous studies reported a population tendency for hypercholesterolemia, one of the main factors contributing to the development of atherosclerosis (AT), considered the primary cause of CAD. Apolipoprotein E has a key role in plasma lipid metabolism, given its function as a ligand for cell-surface receptor mediated uptake of lipoproteins. Polymorphism in the apolipoprotein gene (*APOE*) results in three major isoforms encoded by three codominant alleles (E2, E3 and E4). With the purpose of establishing the pattern of variation at the *APOE* locus and determining its association with lipid profile, we studied a random sample of 298 unrelated, apparently healthy individuals of Azorean origin. In nearly 50% of the sample total cholesterol (TC) was above 200mg/dl; in 25% of the individuals LDL-cholesterol (LDL-C) was higher than 130 mg/dl. Allele frequencies were 0.0833, 0.8317 and 0.0850 for E2, E3 and E4, respectively. Genotype frequencies were higher for E3\*E3 genotype (66.1%); genotype distribution displayed conformity with Hardy-Weinberg expectations. No differences in allelic frequencies were found in comparison with other Caucasian populations, namely with mainland Portugal. E3\*E4 individuals presented the highest cholesterol levels. Analysis of variance performed with the most represented genotypes (E2\*E3, E3\*E3 and E3\*E4) revealed a clear association between the genotypic composition and TC, as well as LDL-C, thus confirming in this population, the role of *APOE* as one of the genetic determinants of AT.

## P17.19

## Genetic risk factors for arterial ischemic stroke in children: a possible MTHFR and eNOS gene-gene interplay?

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Pediatric stroke has become increasingly recognized as an important cause of morbidity and mortality. The etiological heterogeneity of this disease implicates careful consideration of the complex interactions between genetic and acquired risk factors. In order to investigate the influence of genetic factors in childhood stroke, we compared the distributions of mutations/polymorphisms affecting haemostasis and/or endothelial function (FVL Leiden, FIIG20210A, MTHFR C677T, ACE ID and eNOS G894T) among children with stroke and controls. A total number of 26 children with arterial ischemic stroke, and a control group of 50 healthy children were included in the study. No statistically significant differences in allelic and genotypic distribution were detected in comparisons between groups. However, when combined genotypes were analyzed, statistical significance was observed for the association of MTHFR CT and eNOS TT gene variants. The results of our study suggest that this genotype combination represents a risk factor of 7.2 (p=0.017) for stroke in children.

## P17.20

## HMOX1 polymorphism in patients with Atherosclerosis

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Introduction: Heme oxygenase (HO) is important in the defense against oxidative stress and as a factor in an antiatherogenic mechanism. Heme oxygenase (HO) leads to the generation of free iron, carbon monoxide, and bilirubin. A length polymorphism of GT repeats in the promoter of human HO-1 gene shows difference transcriptional activity which modulates the transcription of the gene in vascular cells. The aim of this study was to assess the association of the length of (GT)(n) repeats in the development of coronary artery disease (CAD). METHODS: We screened the allelic frequencies of (GT)(n) repeats in the HO-1 gene promoter in 59 patients who underwent coronary angiography. Because the distribution of numbers of (GT)(n) repeats

was bimodal, based on previous studies we divided the alleles into 2 subclasses: class S included shorter (<27) repeats, and class L included longer (> or =27) repeats. Multivariate logistic regression models including standard coronary risk factors revealed that the genotypes were significantly related to CAD status. In this study, the patients with shorter GT repeats were less likely to have CAD.

**CONCLUSIONS:** Length polymorphism in the HO-1 gene promoter is related to CAD susceptibility in Iranian people who also have coronary disease risk. This study confirms HO-1 antiatherogenic role in Iranian patients with CAD.

#### P17.21

##### Genetic variations in nitric oxide synthase genes NOS1, NOS2A and NOS3 and cerebral small vessel disease

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**Background:** Cerebral small vessel disease (cSVD) is the second most common endemic entity of the ageing brain following Alzheimer pathology. Its hallmark lesions white matter lesions (WML) and lacunar infarctions can be non-invasively depicted with brain MRI. Gait disturbances and progressive cognitive impairment are frequent clinical consequences. Major risk factors are hypertension and age. Its heritability is in the range of 55-73%. Nitric oxide is an important regulator of blood pressure and cerebral blood flow and has been implicated in ischemic stroke. Here we studied genetic variations of nitric oxide synthetases, NOS1, 2A and 3 in relation to cSVD.

**Methods:** The study was conducted in the Austrian Stroke Prevention Study a prospective, cohort study in the normal elderly in Graz, Austria. In total 787 participants underwent genotyping and MRI. Genotyping was done by Illumina Human610-Quad BeadChip. Association was tested by additive genetic model with 1-degree of freedom trend test relating genotype dosage, 0 to 2 copies of the minor allele to WML volume and lacunes. Adjustment was done for age and sex (model1) and for age, sex and hypertension (model 2).

**Results:** We selected 9 tagging SNPs in NOS1, 17 in NOS2A and 12 SNPs in NOS3 gene. Several SNPs in NOS1 and NOS2A gene showed significant associations in both models with WML but not with lacunes. Association was no longer significant after adjustment for multiple comparison.

**Discussion:** Our results do not support the role of SNPs at the NOS1, 2A and 3 genes in cerebral small vessel disease.

#### P17.22

##### NOTCH3 Gene and Cerebral Small Vessel Disease

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**Background:** Cerebral small vessel disease (cSVD) is the second most common entity of the ageing brain following Alzheimer pathology. Its hallmarks are white matter lesions (WML) and lacunar infarctions, detected by brain MRI. Gait disturbances and cognitive impairment are the clinical consequences. Risk factors are hypertension and age. Its heritability is in the range of 55-73%. In the present study we investigated the role of NOTCH3 gene in cSVD. Mutations in NOTCH3 cause CADASIL, a monogenetic form of cSVD.

**Methods:** The study population consisted of 923 participants of the Austrian Stroke Prevention Study a population-based, prospective, cohort study. cSVD was defined by MRI on T2 weighted images. Polymorphisms in the NOTCH3 gene were screened in 88 persons with and 82 persons without cSVD by denaturing HPLC or by sequencing. SNPs (rs1043994, rs10423702, rs1043997) were genotyped in the whole cohort by TaqMan<sup>TM</sup> assay.

**Results:** We detected 35 SNPs in the NOTCH3 gene, 10 SNPs were not described previously. In total 23 SNPs were located in exons, 4 in introns, 3 in the promoter and 2 in the 3'-UTR. There was a non significant difference in the distribution of the SNPs rs1043994, rs10423702 and rs1043997 between cSVD positive and negative subjects.

**Discussion:** This is the first study investigating the whole NOTCH3 gene in healthy population and its role in cSVD. Our results show that SNPs in NOTCH3 gene appear with a high frequency in the elderly. So far our data do not support a role of NOTCH3 in cSVD.

#### P17.23

##### CYP2C9\*3 allele modifies the activity of the renin-angiotensin system in hypertensive men

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CYP2C9 catalyses the formation of epoxyeicosatrienoic acids (EETs) that have been described to show antihypertensive action in kidneys and vasculature. Two variants, CYP2C9\*2 and \*3, have reduced catalytic activity. We studied the impact of these variants on the activity of the renin-angiotensin-aldosterone system (RAAS) in two cohorts of hypertensive subjects.

The GENRES Study consisted of 219 hypertensive Finnish men, aged 35 to 60 years, who were treated with four different antihypertensive monotherapies for four weeks. Baseline laboratory values were measured at the end of the first placebo period. The cohort with treatment-resistant essential hypertension (TREH) consisted of 170 females and 145 males who completed a captopril challenge test (CCT) and had no interfering medications.

Allele frequencies for CYP2C9\*2 and \*3 in both cohorts correspond to reported Caucasian frequencies. In the GENRES group, CYP2C9\*1\*3 genotype was associated with lower baseline plasma renin activity (PRA) and serum aldosterone levels compared with CYP2C9\*1\*1 genotype ( $P=0.0008$  and 0.009, respectively). In the TREH group, PRA and aldosterone levels and their product were lower in male CYP2C9\*3 allele carriers ( $P=0.09$ , 0.19, and 0.04, respectively), and these males had a lower increase in PRA ( $P=0.24$ ) and a lower reduction in serum aldosterone ( $P=0.003$ ) upon CCT. In addition, these males had higher serum sodium levels ( $P=0.08$ ).

In conclusion, this study carried out in two independent cohorts of hypertensive men shows that CYP2C9\*3 influences RAAS activity. These findings might reflect variations in CYP2C9-mediated EET metabolism and call for additional studies on the eicosanoid-related genetic effects in human hypertension.

#### P17.24

##### Deletion allele in a Tunisian healthy and myocardial infarction population

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**Background:** The role of the insertion/deletion polymorphism in the angiotensin-converting enzyme gene (ACE I/D) on myocardial infarction (MI) is controversial. Individuals homozygous for the deletion have a higher level of circulating enzyme and therefore may predispose to cardiovascular damage.

**Aim:** to assess the effect of the ACE polymorphism on MI and its relationship with serum ACE activity and to compare them with other populations.

**Patients and Methods:** 119 patients with MI compared to 380 healthy controls, originated from the same areas, and genotyped by PCR. Serum ACE activity was measured by FAPGG as substrate.

**Results:** The ACE I/D was significantly associated with MI ( $p<0.001$ ). A significant association between the DD genotype and increased risk of MI [ACE DD vs ID and II; OR=3.17 (95% CI, 2.31-4.33;  $p < 0.001$ )] and between II genotype and decreased risk of MI [ACE II vs. DD and ID, OR= 0.33 (95% CI, 0.14-0.47;  $p < 0.001$ )].

Serum ACE activity was significantly higher ( $P<0.05$ ) in patients with MI with the ACE DD genotype ( $99.2 \pm 47.7$  U/L) compared with subjects with the ID ( $70.9 \pm 31.4$  U/L) and the II ( $57 \pm 45.3$  U/L) genotypes.

**Conclusion:** As ACE DD genotype has been associated with increased serum ACE levels, these findings may implicate ACE I/D polymorphism as a genetic marker of MI risk in Tunisian population.

## P17.25

Association study between variants in *GAS6-TAM* genes and atheroma carotid plaque

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Previous studies of our group indicated an association between a SNP and a haplotype of *GAS6* and stroke. Carotid atherosclerosis (CA) is a common cause of stroke and recent studies suggest that pathways initiated by the interaction of the plasma vitamin K-dependent protein *GAS6* with the tyrosine kinase receptors *TYRO3*, *AXL* and *MERTK* (TAM) may have a relevant role in atherogenesis.

The aim of this study was to analyze the genetic association between SNPs and haplotypes in *GAS6-TAM* genes and CA.

We performed a case-control study with 145 patients who had CA confirmed by nuclear magnetic resonance and 162 patients who suffered from cardioembolic (non atherogenic) stroke. For all patient and control samples there was information on traditional risk factors. Genotyping of 19 selected SNPs was performed by real-time PCR, using both FRET or TaqMan probes.

Minor allele frequencies were different between atherogenic and non atherogenic populations for the rs2277537 and rs16971872 SNPs in *TYRO3*. Adjusted logistic regression (LR) analyses indicated that rs2277537 in *TYRO3* and rs869016 in *MERTK* associated to CA, respectively increasing (OR:2.72 [0.95-7.78]) and decreasing (OR=0.39 [0.10-0.79]) the risk of CA. Linkage disequilibrium results were in concordance with the haplotype blocks described in HapMap and adjusted LR analyses revealed that the haplotypes GCTCA in *TYRO3* and the ACAA in *MERTK*, both containing the minor allele of the associated SNPs, were also associated to CA. The association between *GAS6-TAM* SNPs and carotid atherosclerosis reinforce a physiological role of the *GAS6-TAM* pathway in atherogenesis.

## P17.26

The study of *GATA4* gene tagging SNP in patients with arterial hypertension and ischemic heart disease

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Numerous researches demonstrated that calcineurin pathway plays a crucial role in cardiac hypertrophy and progression of heart failure. The aim was to study tagging SNPs in *GATA4* gene in healthy individuals and patients with cardiovascular disease and cardiac hypertrophy.

We selected tagging SNP in *GATA4* gene and analyzed polymorphisms rs804271, rs8191515 in promoter and rs2898293 in intron of *GATA4* gene in patients with essential hypertension (n=155), ischemic heart disease (n=153), hypertension combined with diabetes mellitus 2 (n= 90) and healthy volunteers (n=285).

Genotype frequencies were in accordance with Hardy-Weinberg equilibrium. Frequency of rs804271 rare allele in control group was 47%; rs8191515 - 10%; and rs2898293 - 32%. Patients did not differ from healthy subjects in *GATA4* gene allele and genotypes frequencies. To further analyze the role of genetic variants in cardiac remodelling we divided patients with essential hypertension and hypertension combined diabetes mellitus 2 into subgroups with left ventricular hypertrophy (LVH) and without LVH based on the left ventricle mass index. We had not revealed differences in *GATA4* gene allele and genotypes frequencies between patients with and without LVH.

In our previous studies it was shown that polymorphisms in two genes of calcineurin pathway (PPP3R1 and NFATC4) may be involved in cardiac remodeling in patients with arterial hypertension. It is essential to investigate polymorphisms of calcineurin pathway genes, including tagging SNP, because functional genetic variants in these genes are still unknown.

## P17.27

## Large scale genome wide association study identifies new genetic loci determining homocysteine levels

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Elevated levels of plasma homocysteine (Hcy) are a risk factor for many common clinical conditions, including cardiovascular disease. Inter-individual variation in Hcy levels is highly heritable ( $h^2 \sim 70\%$ ), but the genetic component is poorly understood. We conducted the first genome-wide association study (GWAS) of plasma Hcy levels using four populations from Switzerland, the Netherlands and UK with a total sample size of 11,888 individuals. We identified five loci (seven independent associations) reaching genome-wide significance. Five of the seven associations were present in or near three previous candidate genes: *MTHFR* (rs1801133,  $p=2 \times 10^{-47}$ ; rs11121480,  $p=4 \times 10^{-19}$ ), *CBS* (rs6586282,  $p=5 \times 10^{-12}$ ; rs1789953,  $p=2 \times 10^{-11}$ ) and *MTR* (rs10925257,  $p=2 \times 10^{-9}$ ). We identified two completely novel loci including one on chromosome 16q24.3, rs908951 ( $p=2.1 \times 10^{-11}$ ), located in dipeptidase 1 (DPEP1) and one on 11q14.3, rs7130284 ( $p=9 \times 10^{-11}$ ) in the NADPH oxidase 4 gene (NOX4). Neither gene has any obvious functional link to Hcy, although 16q24.3 also contains the Fanconi anemia gene FANCA and the top hit in this region (rs908951) is strongly associated with FANCA mRNA levels. A strong candidate gene in the 11q14.3 region is the *PSMAL* gene, which is highly homologous to folate hydrolase I, which is pivotal in the uptake of folate from the diet. Collectively, the 7 independent polymorphisms account for up to 4% of the variation in Hcy. We are currently investigating the impact of the 7 variants on cardiovascular disease. Our results provide a new insight into key physiological mechanisms that might underpin many complex diseases including cardiovascular disease and highlight novel therapeutic targets.

## P17.28

## Hunting for young-onset hypertension genes using a genome-wide gene-based association method

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Hypertension has high prevalence and large social impacts in various ethnic populations. Known hypertension-associated genes provide useful but not sufficient information to unravel this complex disorder. We conducted a genome-wide association study with 198 hypertensive patients and 192 normotensive controls of Han Chinese to identify novel disease genes of young-onset hypertension. All samples were genotyped with the Affymetrix Human Mapping 500K Set. A two-stage genome-wide association analysis with a SNP-based association scan at the first stage and a gene-based association scan at the second stage was performed. Logistic regression models with/without adjustments of age, gender and body mass index were fitted to infer SNP marginal effects and then a p-value combination method is applied to study gene effects. Multiple-test problem is corrected by considering a false discovery rate of one-thousandth, i.e.,  $-\log_{10}(FDR) > 3$ . Among 155,706 intergenic SNPs and 14,309 genes, we identify 23 intergenic SNPs and 134 genes, which contain novel genes/SNPs and known genes involved in pathways of cardiovascular disease, metabolic disease, immune disease and cell development. The identified genes cover 2 - 207 intragenic SNPs probed on Affymetrix 500K Set. A gene-expression confirmation study and a SNP-replication study are in preparation.

## P17.29

## Novel polymorphic AluYb8 insertion in hypertension candidate gene WNK1

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Essential hypertension with its concurrent risk to other cardiovascular diseases affects approximately 25% of population in industrialized societies. Determining the genetic component of the disease is crucial for better understanding of the molecular basis of the phenotype and for developing more effective treatment of the disease.

I present the data on a novel, so far non-described human polymorphic intronic *Alu*/*Yb8* element located in hypertension candidate gene *WNK1*. *WNK1* plays an important role in salt homeostasis through different mechanisms and thereby has functional importance in blood pressure regulation. Over-expression of the *WNK1* gene is associated with high blood pressure.

The comparative sequencing showed that the surrounding genomic region of the human-specific *WNK1* *Alu* insertion is highly conserved between human and chimpanzee. Population genetic study targeting the distribution of the *Alu* insertion in 22 human population samples from Europe, Asia and Africa indicated an expansion of the *Alu*-bearing chromosomes in Europe and Asia. The association between the carrier status of the intronic *Alu*/*Yb8* element and blood pressure was studied using 1211 untreated subjects from the HYPEST cohort (Estonian population). Statistically significant association was detected between the carrier status of the *WNK1* *Alu*/*Yb8* and systolic as well as diastolic blood pressure (linear regression testing,  $p = 0.01$  and  $p = 0.03$ , respectively). Subjects with the *Alu* insertion had higher blood pressure readings. Real-time PCR analysis showed that *Alu* insertion affects the profile of alternative *WNK1* transcripts.

In conclusion this study suggests a possible involvement of *WNK1* intronic polymorphic *Alu*/*Yb8* in increasing the susceptibility to essential hypertension.

#### P17.30

#### Association Between Variants in the Genes for Leptin, Leptin Receptor and Proopiomelanocortin with Chronic Heart Failure in the Czech Population

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**Background:** Patients with chronic heart failure (CHF) express enhanced catabolic metabolism resulting in overall weight loss and adipokines are generally recognized to play the crucial role in adipose tissue signaling. The aim of this study was to investigate the possible associations of defined variability in leptin (dbSNP ID rs7799039), proopiomelanocortin (dbSNP ID rs3754860 and dbSNP ID rs1009388) and leptin receptor gene (dbSNP rs1137101) with CHF and evaluate their potential as the CHF susceptibility genes.

**Methods:** The case-control study comprised a total of 372 patients of Caucasian origin with chronic heart failure (functional classes NYHA II-IV, ejection fraction (EF) < 40%) and 407 healthy controls. The subjects were genotyped for the LEP -2548 G/A, LEPR Gln223Arg and POMC RsaI (5'-UTR) and C1032G variants (intron 1) by means of PCR-based methodology.

**Results:** No case-control differences in genotypes or allele frequencies as well as POMC haplotypes were observed between CHF patients and controls. In multivariate regression modeling, the LEPR Gln223Arg showed an independent prediction role for CHF, with the A allele being more frequent in CHF under 56y of age ( $p = 0.0002$ , OR = 1.29, 95% CI = 1.089 - 1.549).

**Conclusions:** Based on our findings, the RR genotype of LEPR Gln223Arg polymorphism might be considered a genetic marker for earlier CHF onset both in ischemic heart disease or dilated cardiomyopathy patients. However, the role of the polymorphic variants in the genes encoding for adipokines as potential CHF susceptibility genes will require further investigation to elucidate the underlying pathophysiological consequences.

#### P17.31

#### Combined effect of the FABP2, PPARG2 and PC1 gene polymorphisms on the metabolic syndrome risk in a Romanian population

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**Objective.** Based on the supposed multifactorial inheritance, we proposed to follow the joint effect of three SNPs in candidate genes of the metabolic syndrome. We have studied the risk of the disease diagnosed according to the IDF recommendations in the presence of the FABP2-A54T, PPARG2-P12A and PC1-K121Q polymorphisms in the population from Tg. Mures.

**Material and methods.** We carried out a case-control study on 144 patients and 73 healthy controls. Anthropometric measurements, biochemical assays were undertaken, fasting insulinemia was measured by ELISA, and insulin sensitivity was assessed by the QUICKI indices. Genetic analysis was done by PCR followed by restriction enzyme digestion with Hha I, BstU I and Ava II.

**Results.** Regardless of their type, the increase of the number of predisposing alleles on the three studied loci associates with a significantly increased risk to develop the syndrome ( $\chi^2$  for trend = 7.99, df = 1,  $p = 0.0047$ ), and the risk was highest in the presence of the TT + PP/PA + QQ/KQ genotype combination (OR = 4.31,  $p = 0.015$ ).

**Conclusion.** Our results confirm the small but additive effect of certain predisposing gene polymorphisms as part of a polygenic system in the development of the common metabolic syndrome, though individual gene combinations can be presumed and other gene interactions like epistasis seem also plausible.

#### P17.32

#### Polymorphic genetic markers are of significance in developing of cardiovascular complications of the metabolic syndrome

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The metabolic syndrome (MS) is a cluster of metabolic abnormalities often associated with development of various complications including cardiovascular diseases. Recently, many studies showed some polymorphisms in apolipoprotein genes might be associated with development of cardiovascular complications of MS. **OBJECTIVE:** to study the association of several polymorphisms in apo-genes APOA1 G-75A, APOA1 C83T, APOC3 Sst1, APO E epsilon, APOA5 T-1131C, and APOA5 S19W with MS complicated with acute coronary syndrome (MS/ACS). **STUDY POPULATION:** MS/ACS patients, among them 76 males and 61 females (average age  $61.7 \pm 1.0$ ), and the controls, among them 114 healthy males (average age  $40.0 \pm 0.5$ ) and 84 females (average age  $85.9 \pm 0.5$ ) without any cardiovascular disease. All of them were investigated clinically, biochemically and genetically. **RESULTS:** Among male MS/ACS patients, there was a lower rate of carriers of APOE e4-allele compared to the controls (18% vs. 28%, OR=0.62, 95%CI 0.38-1.02,  $p=0.075$ ). Also among them, there was a higher rate of carriers of APOA5 19W- allele compared to the controls (18% vs. 3%, OR=6.75, 95%CI 2.01-22.70,  $p<0.0001$ ). No significant difference in genotype distributions of the studied apo-genes was found between the female groups. All studied groups were shown to correspond with Hardy-Weinberg equilibrium. **CONCLUSION:** Our results suggest that the APOE and APOA5 S19W polymorphisms are of significance in male MS patients with ACS.

## P17.33

## Cardiac- and muscle-specific microRNAs miR-1, miR-133 and miR-208 dysregulation in human myocardial infarction

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MicroRNAs (miRNAs) are small RNA molecules that regulate gene expression in a variety of physiological functions, development and disease. They are believed to be new promising therapeutic targets, biomarkers and/or prognostic factors. From 866 human miRNAs described so far, three miRNAs have been described as muscle and/or cardiac specific: miR-1, miR-133, and miR-208, that contribute to heart development and heart diseases. In animal model of myocardial infarction (MI), to the best of our knowledge, there is only miR-1 expression pattern analysed so far. We therefore analysed expression of miR-1, miR-133a, miRNA-133b and miR-208 in human MI and fetal hearts in comparison to the normal adult hearts. Autopsy samples of infarcted heart tissue from 50 patients with MI were included, as well as heart tissue from 8 healthy trauma victims and 8 fetuses that died in utero. miRNAs expression was analysed using real-time PCR. miR-208 was up-regulated in all cases of MI compared to healthy adults and fetuses. Significant miR-208 up-regulation (~4-fold) was detected in MI patients, indicating stress-induced up-regulation, and ~2-fold in MI patient with ventricular fibrillation and/or tachycardia (VT/VF), indicating its contribution to arrhythmogenesis. miR-1 and miR-133 expression analysis suggested time-depended expression pattern in MI, whereas all tested miRNAs were down-regulated in fetal in comparison to adult hearts. In addition, some patterns of miR-1, miR-133a and miR-133b expression in MI were similar in MI and fetal hearts supporting the concept of reprogramming of cardiac genes in remodeling of the heart.

## P17.34

## The left ventricular systolic heart insufficiency in normotensive CAD patients is associated with MTHFR 677TT genotype, whereas in hypertensive APOE 33 subjects with MTHFR 1298CC genotype

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The methylenetetrahydrofolate reductase (MTHFR) 677C>T and 1298A>C genotypes, which influence homocysteine (tHcy) plasma level, were studied for the possible specificity of involvement in development of the left ventricular systolic insufficiency (LVSI) as late effect of coronary artery disease (CAD).

A group of 190 men with CAD diagnosed by coronary angiography at age <60 years was recruited. LVSI was recognized when the left ventricular ejection fraction value was below/equal 40%. Patients were divided into 4 subgroups according to the LVSI and hypertension occurrence: 105 patients qualified to have normal systolic function (44.8% hypertensive) and 85 patients qualified to have LVSI (47.1% hypertensive). The MTHFR and APOE (apolipoprotein E) genotypes were ascertained by PCR-RFLP methods. tHcy and FA levels were measured by immunochemical methods. The differences in distribution of MTHFR genotypes were studied in CAD patients in relation to the APOE33 genotype, hypertension and LVSI cooccurrence.

The subgroup of normotensive subjects with LVSI, as compared to the rest of patients, was characterized by the 2.6-fold higher frequency of 677TT homozygotes ( $p<0.05$ ) and the higher tHcy level ( $15.5 \pm 13.2 \mu\text{mol/L}$ ;  $12.0 \pm 5.4 \mu\text{mol/L}$ ; respectively;  $p=0.04$ ). In the subgroup of hypertensive patients with LVSI, the higher frequency of 1298CC homozygotes was found as compared to the other patients, but this difference was significant only among subjects with APOE 33 genotype ( $OR=3.7$ ;  $p<0.05$ ).

In conclusions, the risk of LVSI development may be induced by different mechanisms by MTHFR 677C>T and 1298A>C genotypes in normotensive and hypertensive men with CAD, respectively. Grant from Ministry of Education N40208131/2499.

## P17.35

## Molecular genetics investigation of myocardial infarction of the Yakut population

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Myocardial infarction (MI) is a complex disease reflecting the interaction of multiple genes with environment. These data show that some single-nucleotide polymorphisms of the candidate genes are associated with myocardial infarction (MI) among the Yakuts.

We have investigated 102 males having myocardial infarction of under 55 years of age and 152 males for control group. Both groups are matched by age. Genomic DNA was extracted from peripheral blood leukocytes. The *APOA* (158Arg/Cys and 112 Cys/Arg), *APOB* (*EcoRI*, *Xba*), *LPL* (*HindIII*), *CETP* (421Ile/Val), *eNOS* (*VNTR*), *PON1* (192Gln/Arg), *ACE* (I/D), *AT1R* (1166A/C) polymorphisms were determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analyses on agarose, and polyacrylamide gel electrophoresis. Statistical tests were performed with the MS Excel XP (Microsoft), "Statistica for Windows 5.0" (StatSoft), "GENEPOP" and "RxC" (Rows x Columns).

Results: The genotype frequencies were consistent with Hardy-Weinberg equilibrium and not significantly different. We have detected associations with MI in Yakut population on *APOE*, *APOB* (*Xba*), *eNOS*, *ACE*, *AT1R* genes polymorphisms. The higher risk of MI showed in *APOB* (*Xba*) genotypes  $X+X+$  ( $P=0.022$ ,  $OR=4.02$ ),  $X+X-$  ( $P=0.022$ ,  $OR=2.02$ ), and allele  $X+$  ( $P=0.0001$ ,  $OR=2.56$ ), in *eNOS* allele *4A* ( $P=0.022$ ,  $OR=2.35$ ), in *AT1R* genotype *A/C* ( $P=0.021$ ,  $OR=2.45$ ). The lower risk of MI have *APOE* genotype *3/3* ( $P=0.042$ ,  $OR=0.55$ ), *APOB* genotype *X-X-* ( $P=0.001$ ,  $OR=0.38$ ), *ACE* genotype *D/D* ( $P=0.063$ ,  $OR=0.54$ ), *eNOS* genotype *4B/4B* ( $P=0.02$ ,  $OR=0.2$ ).

The latter association enables us to use genotyping polymorphisms for prognosis recurrence in the Yakut myocardial infarction survivors and it's necessary for population research.

## P17.36

## Peripheral arterial disease and methylenetetrahydrofolate reductase (MTHFR) 677C&gt;T mutations: a case-control study and meta-analysis

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Hyperhomocysteinaemia is associated with peripheral arterial disease (PAD). Homocysteine metabolism is affected by multiple genetic, dietary and other environmental factors.

This study considered the association of methylenetetrahydrofolate reductase (MTHFR) 677C>T polymorphisms with the incidence of PAD by performing a case-control study and a cross sectional study of homocysteine levels. We recruited 133 patients with PAD in Norfolk (for a folic acid intervention study, FOLCLAUD) and compared the *MTHFR* allele distribution with 457 healthy individuals. We also carried out a meta-analysis to place our data within the context of other published studies. We searched Medline, Embase and Cochrane databases up to March 2008 for any studies on the association between *MTHFR* 677C>T polymorphism and PAD.

The *MTHFR* 677C>T allele frequencies in the cases and controls were 0.37 and 0.33 and the odds ratios for the association of the 677 T allele or TT genotype with PAD were 1.18 [95% C.I. 0.89, 1.58] and 1.99 [95% C.I. 1.09, 3.63]. Homozygotes for the *MTHFR* 677C>T mutation had higher concentrations of plasma total homocysteine, odds ratio 2.82 [95% C.I. 1.03, 7.77] compared to wild types.

Twelve of 72 articles retrieved from the database search reported the prevalence of mutations in PAD patients. A meta-analysis of 9 appropriate studies, including our own, showed that being homozygous for the C677T allele was associated with an increased risk of PAD; pooled odds ratio 1.36 [95% C.I. 1.09, 1.68].

In conclusion, we have found a strong association between raised homocysteine, the TT genotype and PAD.

## P17.37

**Association of the CCTTT repeat polymorphism in *NOS2* (Nitric oxide synthase 2 gene) promoter region with susceptibility to pulmonary arterial hypertension****D. Valverde<sup>1</sup>, T. Piñeiro-Gallego<sup>1</sup>, I. Pereiro<sup>1</sup>, C. Vilariño<sup>2</sup>, A. Baloira<sup>2</sup>,**<sup>1</sup>University of Vigo, Spain, Vigo, Spain, <sup>2</sup>Servicio de Neumología. Complejo Hospitalario de Pontevedra, Pontevedra, Spain.

Pulmonary arterial hypertension (PAH) is a progressive disease in which the proportion of affected women is twice as men, with an incidence of 1/100000-1000000. Familial cases cover 10% of cases and a model of autosomal dominant inheritance with incomplete penetrance and genetic anticipation has been proposed. Mutations in the gene *BMPR2* have been described as responsible for 90% of familial cases, despite the fact that only 20% of the relatives of mutation carriers develop the disease. For idiopathic pulmonary arterial hypertension, mutations in the *BMPR2* gene have been described in a 9-26% of cases. These data support the hypothesis of the existence of other genes involved and the environmental effect on the development of this condition.

Vasodilators such as nitric oxide (NO) and prostacyclin, along with prolonged overexpression of vasoconstrictors such as endothelin (ET-1), not only affect vascular tone but also promote vascular remodelling. Both have been implicated in the pathogenesis of PAH.

NO is an endothelial-derived relaxing factor that is synthesized from L-arginine by nitric oxide synthase. *NOS2* is the major source of NO production and polymorphisms in the *NOS2* gene promoter are thought to regulate its transcription activity.

The aim of this work was to investigate the association between the CCTTT polymorphism of the *NOS2* gene and the susceptibility to PAH.

We analysed that polymorphism in 30 patients of PAH and 50 controls. The data reveals that the shorter forms of the CCTTT repeat were associated with susceptibility of PAH.

## P17.38

**Vitamin D receptor gene polymorphism fokI is associated with severity of atherosclerosis and poor collateralization in angiographically documented CAD patients****H. Saghati<sup>1</sup>, A. Hosseini-Nezhad<sup>1</sup>, B. Larjani<sup>2</sup>,**<sup>1</sup>Bio & Nano Technology Unit of Endocrinology and Metabolism Research Center, Tehran, Islamic Republic of Iran, <sup>2</sup>Endocrinology and Metabolism Research Center of Tehran University of medical sciences, Tehran, Islamic Republic of Iran.

Introduction: Recent studies found a relationship between Vitamin D and atherosclerosis. The aim of this study was to evaluate severity of coronary artery disease (CAD) and collateral development in different genotypes of vitamin D receptor (VDR) gene.

Methods: In a case-control study 114 participants with angiographically documented CAD and normal coronary were recruited. Severity of CAD was defined by the numbers of involved coronary vessels. The modified TIMI scoring was used to grade the collateral development. Whole blood DNA extraction was performed and FokI polymorphism (C27823T) was determined by PCR-RFLP method.

Results: The mean age of participants was 57.01±10.29 years. Single, two and three-vessel were involved in 27.7, 16.9 and 34.5 percent of participants, respectively. Prevalence of genotype FF, ff and Ff were 54.4%, 38.6% and 7% respectively. BMI, FBS, total cholesterol, triglyceride and LDL were higher among ff though only significant about BMI and triglyceride ( $p=0.03$ ). Age and HDL were lower among ff though only significant about age ( $p=0.003$ ). In younger patients (age lower than 55 years) with ff genotype compared with FF genotype had significantly higher poor-developed (25% vs. 0%) and lower well-developed collaterals (0% vs. 4%) ( $p=0.03$ ). Significantly higher severity of CAD also was found among patients with ff genotype (66% of ff vs. 37.5% of FF had three-vessel involvement- $p=0.01$ ) in patients over 55 years old.

Conclusion: Our findings showed that sever CAD and poor collateralization associated with ff genotype of VDR gene. VDR genotyping may be useful as early predictor of poor prognosis of coronary artery disease.

## P17.39

**Genetic variability in the ACE gene region surrounding the Alu I/D polymorphism is maintained by balancing selection in human populations****R. Cagliani<sup>1</sup>, M. Fumagalli<sup>1,2</sup>, S. Riva<sup>1</sup>, U. Pozzoli<sup>1</sup>, G. P. Corni<sup>3</sup>, N. Bresolin<sup>1,3</sup>, M. Sironi<sup>1</sup>,**<sup>1</sup>Scientific Institute IRCCS E. Medea, Bosisio Parini, Italy, <sup>2</sup>Bioengineering Department, Politecnico di Milano, Milan, Italy, <sup>3</sup>Dino Ferrari Centre, Department of Neurological Sciences, University of Milan, IRCCS Ospedale Maggiore Policlinico, Mangiagalli and Regina Elena Foundation, Milan, Italy.

Angiotensin-converting enzyme (ACE) plays a critical role in the maintenance of cardiovascular homeostasis. Extensive research has aimed at identifying ACE genetic variants responsible for variation in enzyme plasma concentrations and associated with human diseases. These efforts have been hampered by the extensive linkage disequilibrium across the gene and the identity and location of the functional polymorphism(s) is at presently unknown.

Here, we characterize the sequence variation and haplotype structure of the ACE gene region surrounding the Alu insertion/deletion (Alu I/D) polymorphism in 4 human populations. We observed high levels of nucleotide diversity, an excess of intermediate-frequency alleles and, at least in African populations, a higher level of within-species diversity compared to interspecific divergence. Analysis of haplotype genealogy indicated the presence of two major clades separated by deep branches with a coalescence time older than 1.5 million years. All these features strongly suggest the action of balancing selection and we verified that the selection signature is restricted to the gene region surrounding the Alu I/D.

Our data therefore imply the presence of a functional polymorphism in the Alu I/D region and illustrate the contribution of evolutionary models to classic SNP-phenotype association approaches by providing information about the localization of candidate functional variants.

## P17.40

**The effect of codon 71 polymorphism in the apolipoprotein B gene on parameters of lipid metabolism in a Serbian school-age child population****T. M. Damjanovic<sup>1</sup>, I. Novakovic<sup>1</sup>, B. Jekic<sup>1</sup>, N. Maksimovic<sup>1</sup>, M. Vukotic<sup>1</sup>, S. Nedeljkovic<sup>1</sup>, S. Simeunovic<sup>2</sup>, L. Lukovic<sup>1</sup>,**<sup>1</sup>Institute of Biology and Human Genetics, Belgrade, Serbia, <sup>2</sup>University Child Hospital, Belgrade, Belgrade, Serbia.

Lipoproteins are vehicles for distribution of plasma lipids and polymorphism in the genes encoding for apolipoproteins could influence the amount of lipid in plasma. We examined the effect of single nucleotide polymorphism in the codon 71 of the apolipoprotein B (apoB) gene on levels of triglycerides and cholesterol in school-age child population. Studied group included 530 Serbian children attending eight class of elementary school. In all participants standard clinical examination was performed. Levels of total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides were determined by standard biochemical methods. Polymorphism in apoB codon 71 was genotyped by PCR amplification of specific fragment of genomic DNA followed by digestion with the Apa LI restriction enzyme. We found wild type genotype CC in 265 (50%), heterozygous CT in 212 (40,0 %) and homozygous mutant genotype TT in 53 (10,3%) children. Triglycerides level was higher ( $p=0.0673$ ) in TT than in two other genotype groups; this difference was statistically significant ( $p=0.0305$ ) in boys. Also, apolipoprotein (a) level was significant higher ( $p=0.018$ ) in TT group (boys  $p=0.040$ ). Among child with arterial blood pressure under p25 for age carriers of T allele had statistically significant higher total cholesterol ( $p=0.0078$ ), LDL cholesterol ( $p=0.033$ ) and triglyceride ( $p=0.0036$ ) levels comparing to the group without T allele. The increased arterial blood pressure was associated with the significant decrease of HDL cholesterol levels ( $p=0.0249$ ), especially in children with T allele. Our study showed that apoB codon 71 polymorphism has effect on parameters of lipid metabolism in school-age child.

**P17.41****Study for association between the polymorphism T/C of the gene CYP17 promoter and the risk of premature coronary artery disease**C. Agiannitopoulos<sup>1</sup>, H. Kasparian<sup>2</sup>, V. Votreas<sup>2</sup>, K. Lamnisou<sup>1</sup>;<sup>1</sup>Department of Biology, University of Athens, Athens, Greece, <sup>2</sup>Department of Cardiology, "Laiko" Hospital, Athens, Greece.

**Objective:** It is well documented that sex hormones influence the risk of developing cardiovascular disease. Several genes are involved in the synthesis of sex hormones. The CYP17 gene encodes the enzyme cytochrome P450c17 $\alpha$  which functions at key steps in the production of human sex steroid hormones. A T/C polymorphism in the 5' promoter region of the CYP17 gene has been described. In the present study, we investigated the possible association between the T/C polymorphism of the promoter of CYP17 gene on risk of premature coronary artery disease (CAD) in the Greek population.

**Methods:** A total of 180 CAD patients, documented by coronary angiography, aged less than 58 years and 120 healthy controls were studied. To genotype the subjects we used the PCR-RFLP method.

**Results:** The frequencies of GG, GC, CC genotypes were 0.43, 0.38, 0.19, respectively, in the patient group and 0.32, 0.47, 0.21, respectively, in the control group. The frequencies of T and C alleles were 0.62 and 0.38 in the patient group and 0.55 and 0.45 in the control group. The data between the two groups were analyzed by chi-square test. Our results showed that there are no patient/control significant differences.

**Conclusion:** The results of this study suggest that there is no association of the T/C promoter polymorphism of the CYP17 gene with the risk of premature coronary artery disease. Thus, this polymorphism may not be used as genetic marker for CAD risk assessment.

**P17.42****Analysis of GCKR and ApoA5 genes in Hungarian patients with ischemic stroke**L. Járomi<sup>1</sup>, V. Csöngyi<sup>1</sup>, E. Sáfrányi<sup>1</sup>, B. Faragó<sup>1</sup>, L. Magyari<sup>1</sup>, K. Horváthovics<sup>1</sup>, A. Maász<sup>1</sup>, C. Sipeky<sup>1</sup>, Z. Szolnoki<sup>2</sup>, B. Melegi<sup>1</sup>;<sup>1</sup>Department of Medical Genetics and Child Development, University of Pécs, Pécs, Hungary, <sup>2</sup>Department of Neurology and Neurophysiology, Pándy Kálmán County Hospital, Gyula, Hungary.

The development of ischemic stroke is caused by both environmental factors - especially triglyceride level increase - and complex genetic predisposition. Recently, several studies confirmed the role of Glucokinase-hexokinase 4-regulator (GCKR) gene in triglyceride accumulation in patients with coronary artery diseases. The aim of our study was to investigate the genetic association between ApoA5, GCKR gene polymorphisms and the possible changes of triglyceride level in Hungarian ischemic stroke patients. The genotype of the 513 Caucasian patients (207 males, 306 females; mean age: 65.1 $\pm$ 0.62) stratified into three subgroups ("small-vessel occlusion type", "large-vessel group" and "mixed group") and 172 controls (49 males, 123 females; mean age: 56.5 $\pm$ 1.20) was determined by PCR-RFLP methods. The T-1131C, IVS3+G476A and C56G variants in ApoA5 gene increased significantly the level of triglyceride, and associated with higher risk for the development for stroke disease. By contrast, in the case of T1259C in ApoA5 gene and the two polymorphisms of GCKR gene (rs1260326, rs780094) we could not detect significant differences between the stroke patients and the control subjects. In conclusion, we observed a significant association between the disease and elevated triglyceride levels in the presence of three variants of ApoA5 gene, but no correlation was found for the two polymorphisms of the GCKR gene in the Hungarian ischemic stroke population.

**P17.43****Variations in the glucokinase gene and its receptor are simultaneously associated with higher fasting glucose and lower triglyceride**M. Sotos-Prieto<sup>1,2</sup>, M. Guillen<sup>1,2</sup>, P. Guillen-Saiz<sup>1,2</sup>, O. Portoles<sup>1,2</sup>, D. Corella<sup>1,3</sup>;<sup>1</sup>CIBER Fisiopatología de la Obesidad y Nutrición, Valencia, Spain, <sup>2</sup>Department of Preventive Medicine, Valencia, Spain, <sup>3</sup>Department of Preventive Medicine, Valencia, Austria.

The Glucokinase gene (GCK) is a regulator of glucose storage and disposal in the liver and its activity is modulated by binding to glu-

cokinase regulatory protein (GCKR). Studies from genome-wide association (GWA) have identified the SNP rs1260326 at the GCKR gene as a genetic marker for triglyceride concentrations. Moreover, the SNP rs1799884 at the GCK gene has been associated with increased type 2 diabetes risk (DT2) and lower TG concentrations. However, the effects of these genetic variants in the Mediterranean population remain to be investigated. Therefore, our aim was to analyze the effects of GCKR rs1260326 (P446L) and the GCK rs1799884 -30G>A on fasting triglycerides and DT2 in a high cardiovascular risk Mediterranean population. We studied 945 subjects (340 men and 605 women) with high cardiovascular risk (age: 67 $\pm$ 6 years) recruited in Valencia, Spain. The genotypic frequencies of GCKR rs1260326 were 29.8%CC, 51%CT, 19.3%TT, and 64.9%GG; 32.2% GA, 2.9% AA for GCK rs1799884. The TT homozygous had significantly higher triglycerides (CC: 111.4; CT: 124.22; TT: 126.23 mg/dl; p= 0.026) and a borderline lower fasting glucose (p=0.060). However, homozygous subjects (AA) for the promoter SNP had higher plasma glucose and lower triglyceride. Furthermore, the GCK -30AA was significantly associated with increased DT2 risk (adjusted OR: 2.05 [CI: 1.25-3.38]; p=0.005) and the GCK TT with higher hypertriglyceridemia risk (adjusted OR: 1.39 [CI: 1.11-1.74]; p=0.005). Conclusion: GCKR and the GCK genetic polymorphisms are significant determinants of triglyceride concentration and fasting glucose in a high cardiovascular risk Mediterranean population.

**P17.44****PPAR-alpha gene polymorphism correlates with lipid and glucose levels, physical activity and body mass index in children from families with history of cardio-vascular disease.**

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**BACKGROUND:** Children whose parents suffered from cardiovascular disease (CVD) have hypoalphacholesterolemia quite often. Alpha cholesterol levels are regulated by PPAR-alpha. It is well known that HDL cholesterol concentration is regulated by physical activity concentration. **OBJECTIVES:** To study an association between PPAR-alpha gene polymorphism and lipid and glucose levels, physical activity and body mass index (BMI) in children from families with a history of CVD. **STUDY POPULATION:** 58 children (average age 13.2 $\pm$ 0.53) and adolescents with family history of CVD. **METHODS:** We have evaluated physical activity index per week and per day. Lipid spectrum in all patients was determined by enzyme-linked immunoassay. In all patients we detected glucose concentration and BMI. Molecular testing of PPAR-alpha gene polymorphism (L162V) was performed by PCR. **RESULTS:** Among 58 children and adolescents, 53 of them had LL-genotype and 5 had LV-genotype. Frequency of L-allele and V-allele were estimated to be 0.96 and 0.04, respectively. In children carried LV-genotype, we detected a higher level of cholesterol (5.08 $\pm$ 0.08, 4.82 $\pm$ 0.13 mmol/l) and lower triglycerides level (0.84 $\pm$ 0.04 vs 1.49 $\pm$ 0.116 mmol/l) compared to children with LL-genotype. We determined correlations between cholesterol and LDL level ( $r=0.82$ ) in children with LL-genotype and between physical activity intensivity and glucose level ( $r=0.84$ ) in children with LV-genotype. Also we have found a correlation between BMI and glucose level ( $r=0.82$ ) and between HDL and LDL levels ( $r=-0.98$ ) in children with LV-genotype. **CONCLUSION:** children from families with history of CVD carried LV-genotype need both an intensive physical activity and their lipid and glucose levels to be controlled.

**P17.45****Combined genetic variants 6A/6A of MMP-3 and I/I of ACE have a strong preventive evidence in patients with dilatative pathology of ascending thoracic aorta**G. Sinkunaite<sup>1</sup>, A. Tamasiunas<sup>2</sup>, M. Jonikas<sup>1</sup>, R. Benetis<sup>3</sup>, V. Lesauskaite<sup>1</sup>;<sup>1</sup>Laboratory of Molecular Cardiology, Institute of Cardiology, Kaunas University of Medicine, Kaunas, Lithuania, <sup>2</sup>Department of Preventive Medicine, Institute of Cardiology, Kaunas University of Medicine, Kaunas, Lithuania, <sup>3</sup>Department of Cardiac, Thoracic and Vascular Surgery, Kaunas University of Medicine, Kaunas, Lithuania.

Numerous studies have proved impact of genotypes 5A/5A of MMP-3 and D/D of ACE for cardiovascular diseases development. We have

found genetic variants of *MMP-3* and *ACE* genes that might be considered to have a protective effect.

The aim of the study was to evaluate combined effect of 5A/6A genotypes of *MMP3* and I/D genotypes of *ACE* genes in development of dilatative pathology of ascending thoracic aorta (DPATA).

**Material and methods.** We studied 94 (68 males, 25 females) patients with DPATA, the age ranged from 31 to 81 years (median, 64 years) and a random sample of the population consisting of 248 males and 317 females aged 45 to 72 years (median, 60 years) all from Lithuania. Analysis was done on DNA using conventional and real time PCR to genotype polymorphism 5A/6A at a position -1171 of the *MMP3* gene promoter and polymorphism I/D of the *ACE* gene.

**Results.** It was tendency of higher prevalence ( $p=0.09$ ) of *MMP-3* promoter 5A/5A and *ACE* D/D genotypes in the patients with DPATA compared to the persons from the random sample of population, 8.51% and 5.13%, respectively. We did not find any carrier of combined *MMP-3* promoter 6A/6A and *ACE* I/I genotypes in patients with DPATA. In contrast, 7.61% of the random sample population have this combined gene variant. The resulting  $p$  value was  $p=0.003$ .

In conclusion, the frequency of *MMP-3* promoter 6A/6A and *ACE* I/I genotypes might be considered as a gene combination protecting from development of dilatative pathology of ascending thoracic aorta.

#### P17.46

#### Characterization of potential splice site mutations in the LDL receptor gene in patients with clinical diagnosis of Familial Hypercholesterolaemia

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Familial Hypercholesterolaemia (FH) is an inherited disorder characterized by a high concentration of serum low-density lipoprotein (LDL) cholesterol since birth, leading to early development of atherosclerosis and coronary heart disease. FH is most commonly caused by mutations in *LDLR* gene. About 6% are located near a splice junction or within intron, predicted to affect the correct splicing of the mRNA.

A total of 11 potential splice site mutations, were found in the "Portuguese FH Study". Six alterations, five of them novel, needed further investigation. RT-PCR of *LDLR* mRNA isolated from fresh blood mononuclear cells of each patient was performed to test the effect of the alteration on splicing. Three of these variants (c.1060+1G>A, c.2140+1G>A, c.2547+1G>A) caused exon/exons skipping; c.190+4 insTG caused retention of two nucleotides of intron 2, and c.818-2G>A caused retention of 10 nucleotides of intron 5. One variant could not be tested experimentally, but it almost certainly affect splicing because disrupts the invariant GT donor splice sites (1845+1delG). All these alterations lead to a premature termination of the protein which means that the protein coded by the affected allele is smaller and has no function, most possible is degraded within the cell. To correctly estimate the number of transcripts that are not correctly spliced, real time PCR should be performed. This will be the next experimental step.

It is important to functional assay the effect on splicing of the alterations found to prevent genetic misdiagnosis of FH patients and to determine the severity of the disease.

#### P17.47

#### Familial hypercholesterolemia and premature heart disease

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Familial Hypercholesterolemia (FH) is an autosomal dominant disorder, usually caused by mutations on *LDLR*, *APOB* and *PCSK9* genes which is associated with premature atherosclerosis and premature coronary heart disease (CHD).

Objective: To assess the ability to achieve clinical and molecular char-

acterization of patients and relatives with family history of premature CHD.

**Material And Methods:** A clinical questionnaire of the "Portuguese FH Study" was filled for all patients which included the characterization of premature CHD and lipid profile. Mutations in *LDLR*, *APOB*, and *PCSK9* genes were analysed by PCR amplification and sequencing, in a retrospective and prospective study including 80 index cases (IC) and 110 relatives.

**Results:** A total of 22/190 (11.57%) patients, 14/80 (17.5%) IC and 8/110 (7.27%) relatives, had premature CHD, (15M/1F). The mean total cholesterol (CT) was  $342.39 \pm 69.46$  mg/dl, before treatment (BT) and  $269.28 \pm 22.09$  mg/dl, after treatment (AT). The mean LDLC was  $274.15 \pm 57.48$  mg/dl (BT) and  $190.94 \pm 16.32$  mg/dl (AT). Five novel mutations resulting in a splicing error were identified: (c.2547+1 G>A), (c.2140+5 G>A), (c.1359-5C>G), (c.190+4insTG), and 1016\_1017insG (L318fsX336). These mutations were characterized functionally by *LDLR* mRNA studies. The other mutations are: c.-42C>G, (EX16\_18 del), c.1085delA (D341fsX348), c.530C>T (S156L) and a compound heterozygous [c.670G>A (D203N)] + [c.2146G>A (E695K)].

**Conclusion:** Male patients with *LDLR* mutations present a more severe phenotype since they develop premature CHD more often than women. The early identification of FH patients can prevent the development of premature CHD if patients receive appropriate pharmacological treatment.

#### P17.48

#### Portuguese FH Study: the genetic screening of Familial Hypercholesterolemia in Portugal

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Familial Hypercholesterolemia (FH) is an autosomal dominant disorder with a frequency of 1/500 in most European countries and Portugal should have about 20000 cases. FH is clinically characterized by high levels of plasma cholesterol, leading to premature atherosclerosis and coronary heart disease (CHD).

The aim of the Portuguese FH Study is to identify FH patients in order to prevent the development of premature CHD.

The genetic diagnosis is based on molecular study of *LDLR*, *APOB* and *PCSK9* genes including techniques such as PCR, DHPLC, automated sequencing and MLPA. Blood samples were collected from 343 index patients with clinical diagnosis of FH and 686 affected/unaffected relatives. A total of 320 individuals were identified with a genetic defect in one of these genes: 315 heterozygous, 3 compound heterozygous and 2 true homozygous. Sixty eight different mutations were detected in *LDLR* gene, 38 previously described and 30 exclusive of Portuguese population, including 12 missense mutations, 1 nonsense, 8 splice site mutations, 7 small deletion/insertion and 3 large deletions. *APOB* 3500 mutation was identified in 9 individuals and one carried a non described mutation in *APOB* gene. One novel mutation in *PCSK9* gene was identified in three individuals.

Although the Portuguese FH Study only identified until now 1,7% of FH cases estimated to exist in Portugal, the genetic diagnosis and counselling of these patients should result in the establishment of appropriate treatment and adoption of a healthier lifestyle, allowing them to obtain a quality and life expectancy similar to a healthy person.

#### P17.49

#### The molecular diagnostics of familial hypercholesterolemia in Czech population

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Familial hypercholesterolemia (FH) is an autosomal dominant disorder caused by mutations in the gene encoding the low density lipoprotein receptor (*LDLR*). The frequency of heterozygotes is 1/500. The frequency of homozygotes or compound heterozygotes is 1/1 000 000. Mutations in the *LDLR* gene were determined using exon by exon screening methods based on individual exon amplification, DHPLC analysis and sequencing. We found 14 novel causal mutations

in Czech population.

However, many patients (about 50%) with a clinical diagnosis of FH have no LDLR mutations identified by this approach. Part of the diagnostic gap is attributable to the genetic heterogeneity of FH. Another possible explanation is that mutation analysis using exon by exon screening may fail to detect the mutant allele in case of large intragenic rearrangements avoiding primer annealing in the deleted area. In order to explore the possibility that whole exon deletions or duplications could be the cause of the mutant phenotype, we used MLPA technique in this study. Six different large deletions and three duplications were found in Czech population.

We designed the APEX (arrayed primer extension) based genotyping microarray for the simultaneous detection of 160 selected mutations and 330 bp of resequencing area of the LDLR gene. The first validation results indicate that the FH chip seems to be a suitable tool for the first line screening of mutations in the LDLR gene.

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#### P17.50

##### Mitochondrial DNA haplogroups and the risk of Parkinson disease in a cohort of patients from South Italy

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There is increasing evidence that European mitochondrial DNA (mtDNA) haplogroups J and K, and their shared 10398G single-nucleotide polymorphism (SNP) in the ND3 gene, have a protective role in the cause of idiopathic Parkinson's disease (PD). We tested whether certain polymorphisms in mtDNA can predispose to PD in a population that is rather genetically homogeneous. Here, we determined the distributions of these mtSNPs, the constructed haplogroups and the clusters or superclusters of these haplogroups in a large cohort of 363 PD patients (213 males and 150 females, age at onset  $\geq 50$ ) from South Italy with idiopathic PD vs a group of 400 unaffected individuals from the same geographic area.

In a preliminary analysis we found no mtSNP or mtDNA haplogroup that predisposes to PD in our South Italy population. Specifically our results indicated that the distributions of the nine major European haplogroups among South Italy PD patients did not differ significantly from those of the surrounding European genetic landscape. In particular, from the present study it can be deduced that no mtDNA variations predisposing to PD are present in the South Italy population, and that these results are in keeping with previous studies that failed to detect causally related mtDNA sequence variations in this disorder.

#### P17.51

##### Ceruloplasmin gene variations and Parkinson's disease: an association study in Southern Italian population

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Ceruloplasmin (CP) regulates iron levels in the central nervous system and prevents free radical injury. Iron-related oxidative stress is an important component of the neurodegenerative process in Parkinson's disease (PD). A possible involvement of ceruloplasmin in the pathogenesis of PD has been supported by immunohistochemical studies that reveal a colocalization of ceruloplasmin and Lewy bodies. It has been reported that there are alterations in ceruloplasmin concentration and ferroxidase activity in serum from heterozygous PD patients. Some functional findings show that altered activity of ceruloplasmin may present a vulnerability factor for iron induced oxidative stress in PD. To elucidate the role of this gene in our population, we screened a total of 230 sporadic PD patients and 231 controls from Southern Italy for the six known variations (I63T, P477L, D544E, T551I, R793H and T841R) identified in a German population. All the patients were diagnosed with idiopathic PD according to the UK brain bank criteria and

gave informed consent according to the declaration of Helsinki. The genetic screening was performed by direct sequencing of the coding region of CP gene for four variations and by restriction analysis for the other two variations. Each variation taken individually, did not show association to PD and no significant differences were observed between cases and controls. Our results do not support a possible association between Cp variations and PD in Southern Italian population.

#### P17.52

##### Association study between HFE, TF, TFR genes and Parkinson's disease

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Iron overload increases oxidative stress and may lead to neurodegenerative disorders like Parkinson's disease (PD). Alterations of iron-related genes, therefore, might be involved in the pathogenesis of PD. The aim of this study was to investigate a possible association between the polymorphisms C282Y and H63D of the haemochromatosis (HFE) gene and the prevalence of PD in Southern Italy. These variants of the HFE gene cause an iron overload disorder, known as hereditary haemochromatosis. Contradictory evidence exists on the role of the HFE variants as risk factors for PD. The HFE protein is thought to interact with the transferrin receptor (TFR), lowering its affinity for iron-bound transferrin (TF). Subsequently we also analyzed if the polymorphisms G258S of the TF gene and S82G of the TFR gene are other risk factors for PD. We examined those four polymorphisms in 181 sporadic PD patients and 180 controls from Southern Italy. The clinical diagnosis of PD was based according to the UK PD Brain Bank Society criteria. We carried out a genetic analysis by standard PCR and restriction digestion method. We did not find significant differences in genotype and allele frequencies between PD and controls for all polymorphisms studied. Our results suggest that the C282Y and H63D variants do not contribute significantly to the risk of PD. Furthermore, there was no association between the G258S TF gene and S82G TFR gene with PD. However, it would be interesting to extend our study to other iron related genes to verify their potential role in PD.

#### P17.53

##### Sequence analysis of mtDNA in Parkinson's disease patients from Bashkortostan Republic of Russia

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MtDNA common variation is reported to modify the risk of Parkinson's disease (PD). We evaluated the impact of the mtDNA variant on PD risk in 157 unrelated Tatar PD patients and 183 unrelated control subjects. Whole mtDNA sequencing in two PD patients with haplogroup H (H and H2) and two with haplogroup U (UK1 and UK4) PD patients showed that haplogroup H mtDNA harbored one potentially functional variant, tRNALys A8343G. The Tatar haplogroup H2 mtDNA harbored another unusual variant, a homoplasmic ND2 gene missense mutation (A64T, CI = 82%), which could increase the risk of developing PD. The haplogroup U mtDNAs, UK4 and UK1, both harbored the defining haplogroup U tRNALeu(CUN) variant A12308G and the 16S rRNA A1811G subhaplogroup U variant. They also shared common variants A1438G in the 12S rRNA and A2706G in the 16S rRNA. The UK4 mtDNA had the defining cytochrome *b* gene variant T15693C (M316T). However, this mtDNA also harbored the rare ND4L missense mutation at C10654T (A62V) (CI = 3%) and the polymorphic double missense variant at T8567C in the ATP8/ATP6 gene overlapping region (S68P/I14T) (CI = 23% and 33%, respectively). The UK1 mtDNA had the defining subhaplogroup polypeptide missense mutations ATP6 nt G9055A (A177T), ND3 nt A10398G (T114A), and cytochrome *b* nt T14798C (F18L). However, the UK1 mtDNA also contained a heteroplasmic missense mutation in the COII gene at nt G7637G/A (E18K) (CI = 100%).

It almost certainly caused a functional defect in complex IV and thus contributed to the PD development.

#### P17.54

##### Genetic association of Homer1 gene and Levodopa induced dyskinesia

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Levodopa-induced dyskinesia is a frequent and disabling side-effect observed during the treatment of Parkinson disease.

Homer is a postsynaptic density protein localized at excitatory synapses that interacts with the C-terminal intracellular domain tail of group 1 metabotropic glutamate receptor through a PDZ-like domain. Homer1 is an immediate early gene (IEG) that is regulated by antipsychotics. The aim of this analysis is to study the association between levodopa-induced dyskinesia and Homer1 gene variants.

The study population consisted of 129 unrelated patients from Southern Italy recruited through the Institute of Neurology in Cosenza, Italy (64 males and 65 females) affected by idiopathic PD. The mean age at the time of the assessment was 67+/-8.3, the age of onset of Parkinson was 56.4+/-9.6 and the mean score for the UPDRS was 49.3+/-19.4 in the overall sample.

We have analyzed three promoter markers in HOMER1 gene (rs10942891, rs4704560, rs4704559).

The association with levodopa-induced dyskinesia was analyzed with UNPHASED 3.0.9 regarding allele, genotype and haplotype. Haplotype Odds-Ratio was calculated using the specific test haplotype.

The SNP rs4704559 in Homer1 gene was associated with high incidence of levodopa-induced dyskinesia ( $p<0.05$ ).

#### P17.55

##### The G399S and A141S variants in HTRA2 are not associated to Parkinson's disease in the Spanish population

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Many studies supported a role for mitochondrial dysfunction as cause of the neurodegeneration in some patients with Parkinson's disease (PD). Recently, the possible involvement of the mitochondrial serine protease HTRA2 gene in the pathogenesis of PD was evaluated in different populations. Two variants (p.G399S and p.A141S) were first reported as pathogenic mutation and susceptibility polymorphism, respectively, in German PD patients. However, the lack of confirmation of this association in other ethnic groups opened up a controversy regarding the role of HTRA2 in PD. Here, we present the results of the screening of exons 1, 2, 7 and 8 of HTRA2 in 290 PD patients (56% males, mean age 69.09 ± 10.08) and 219 healthy controls (47% males, mean age 75.16 ± 6.81) from Spain. We found no difference in allele frequency of p.G399S between patients and controls (MAF-patients = 0.52, MAF-controls = 0.46,  $P = 1$ , OR = 1.134, CI 95% = 0.19-6.85). Similarly, an association of the p.A141S variant to PD was not replicated in the present study (MAF-patients = 2.07, MAF-controls = 0.91,  $P = 0.201$ , OR = 2.320, CI 95% = 0.73-7.29). Analysis of nearby SNPs supported that the T allele of the p.A141S variant in our population lays within the same rare haplotype as reported for the Belgian population. Additionally, we found some novel sequence variants in HTRA2, all of which were present in controls. In view of this data, the contribution of HTRA2 and particularly the p.A141S variant to the risk of PD is unlikely.

#### P17.56

##### Novel splice site mutation in intron 9 of park2 observed in an Iranian patient affected with Parkinson's disease

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Parkinson's Disease (PD) is the second most common neurodegenerative disease. PD is considered a complex disease, and both genetic and environmental factors are relevant to its etiology. Several genes associated with PD have been identified by linkage analysis in multi-case families. PARK2 on chromosome 6, the second largest human gene reported to date, is one of the PD associated gene. Lesions in this gene have most often been observed in familial and young onset cases. PARK2 encodes Parkin, a protein of 456 amino acids that is an E3-ubiquitin ligase, a critical component of the pathway that covalently attaches ubiquitin to a protein targeted for degradation. We sequenced the PARK2 gene in 96 unrelated Iranian PD patients. A novel variation was observed in intron 9 (IVS9+1; c.1083 +1 G>A) of one patient in the homozygous state. The variation affects a highly conserved position in the 5' splice site of nucleus gene. Further analysis revealed the presence of the same variation in the homozygous state in the patient's affected sister. The variation was observed neither in 220 controls nor in 140 additional patients as assessed by ARMS-PCR. These observations are consistent with the proposal that the novel variation G IVS 9 +1 A in PARK2 can cause Parkinson's disease.

#### P17.57

##### Analysis of the $\alpha$ -synuclein gene dosage in autosomal dominant Parkinson's disease

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Parkinson's disease (PD) is the second most common neurodegenerative disorder, characterized by the progressive loss of dopamine neurons and the accumulation of Lewy bodies. Familial autosomal dominant PD accounts for up to 15% of all PD cases. The  $\alpha$ -synuclein (SNCA) gene was the first to be associated with familial autosomal dominant PD. Besides three point mutations, a number of the SNCA gene duplications and triplications have been detected. The objective of this study was to assess the frequency of SNCA multiplications among autosomal dominant PD patients from Russia. We screened a group of 52 autosomal dominant PD patients for duplications and triplications of exons 4-6 of the SNCA gene by TaqMan real-time PCR. The analysis revealed no increase in exon dosage. Our results imply that duplications and triplications of the SNCA gene play insignificant part in the pathogenesis of autosomal dominant PD in Russia.

#### P17.58

##### Young and Late onset Parkinson's Disease: Insights from $\alpha$ -synuclein and more

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Objectives: Sporadic Parkinson's disease (PD), often believed to be a disease of the elderly (late onset, LOPD, age of onset > 40yrs), however has ~ 10% cases with young onset (YOPD, onset < 40yrs). While tremors and rapid progression of disease is seen in LOPD, patients with YOPD often show dystonia and slow progression. We investigated the role of polymorphisms in known PD genes ( $\alpha$ -synuclein, Parkin, PINK1 and DJ1) in understanding the disparity in age of onset, symptoms and disease progression among YOPD ( $n_{cases} = 140$ ,  $n_{controls} = 154$ ) and LOPD ( $n_{cases} = 347$ ,  $n_{controls} = 320$ ). Based on our results, functional characterization of variants in regulatory regions of  $\alpha$ -synuclein are being explored to elucidate their role in gene expression and PD etiology.

Results: Significant associations (corrected for multiple corrections  $\alpha=0.001$ ) of Parkin IVS7-35G>A [OR (95%CI) = 2.52(1.49-4.248) for AA] with YOPD and Parkin promoter (-258T>G) [OR (95%CI) = 2.05(1.46-2.88) for TG] with LOPD were observed. Further, 3'UTR SNP (rs356165) of  $\alpha$ -synuclein was found associated with both YOPD

[OR (95%CI) =3.47 (1.92-6.31) for CC] and LOPD [OR (95%CI)= 2.07 (1.43-2.99) for CC]. Promoter, using luciferase reporter gene assay and 3' UTR variants in *α-synuclein* is currently under investigation.

Conclusions: Similar to rare mutations, SNPs in regulatory regions of known PD genes may confer susceptibility to PD. While some SNPs show age specific associations [Parkin IVS7-35G>A with YOPD; and Parkin promoter (-258T>G) with LOPD], others (*α-synuclein* rs356165) seems to have a role in etiology of both YO and LOPD. Functional characterization of variants in *α* synuclein regulatory region(s) may provide additional insights.

#### P17.59

#### Genetic analysis of SCA2 and SCA17 in familial Parkinson's disease

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Spinocerebellar ataxias (SCAs) refer to a group of neurodegenerative diseases characterized by cerebellar dysfunction alone or in combination with other neurological abnormalities. These disorders link to more than 20 genetic loci. These diseases are often caused by expansion of triplet repeats encoding polyglutamine tracts. Parkinson's disease (PD) has been related to mutations associated with SCAs. The aim of this study was to investigate a selected group of familial PD patients and healthy controls through genetic analysis of SCA2 and SCA17 genes. The patients did not carry either SNCA (A30P, A53T, E46K) or UCH-L1 (I93M) mutations and were negative for LRRK2 G2019S and I2020T.

Eighty-five PD unrelated patients with autosomal dominant inheritance, belonging to southern Italian families with at least three affected members over three generations, and 100 controls were analyzed for CAG expansions in the SCA2 and SCA17 genes. PCR products, amplified with fluorescent primers spanning the SCA expansions, were separated onto a capillary ABI3130XL sequencer and analyzed by the software Genemapper.

SCA17 mutations were detected in 2 (2.3%) of the examined patients, whereas no cases were positive for SCA2. Neither SCA17 nor SCA2 expansions were identified in controls. The size of CAG repeats in SCA17 was small (43-44 repeats, usually associated with reduced penetrance)

Our results show that SCA17 is a rare genetic cause of PD in our population. However, after exclusion of genetic mutations of other known PD genes, SCA17 should be taken into account for the molecular diagnosis of familial PD with autosomal dominant inheritance.

#### P17.60

#### Alfa-synuclein point mutation analysis in Italian patients with autosomal dominant Parkinson disease

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α-synuclein is the major component of Lewy Bodies (LB) and Lewy neurites, the pathological hallmarks of sporadic Parkinson Disease (PD) and Dementia with Lewy Bodies (DLB).

Point mutations in the SNCA gene are very rare and have been identified in few families with autosomal dominant inherited PD.

In this study we screened SNCA exons 2 and 3 by direct sequencing to examine whether SNCA more common point mutations were present in our cohort of patients. All PD patients belonged to a single Italian clinical centre (Parkinson Institute - I.C.P., Milan, Italy - <http://www.parkinson.it/dnabank.html>).

One hundred forty-four unrelated PD patients with dominant PD family history were included in the study. They all had at least one parent with a clear diagnosis of PD. Since SNCA gene rearrangements (triplification as well as duplication) of the entire gene have been confirmed to be the cause of autosomal dominant PD, all these patients were first analyzed using the MLPA (Multiplex Ligation-dependent Probe Amplification) assay Kit "SALSA P51". One patient was found to carry

the SNCA gene duplication and thus was excluded from the sequencing screening. All PD patients were previously tested for the G2019S-LRRK2 mutations and 8 were found to be carriers. Nevertheless these patients were not excluded from the SNCA analysis.

Up to now we have found one SNCA mutation, the p.A53T.

#### P17.61

#### GIGYF2 (TRNC15) mutation analysis in patients with familial Parkinson's disease with autosomal-dominant transmission

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A study has provided a strong support for a role of mutations in the GIGYF2 gene (PARK11) as frequent cause of familial Parkinson disease (Lautier et al, 2008). The aim of this study is to perform mutational analysis of the GIGYF2 gene in patients with familial PD with autosomal dominant transmission, that have resulted negative by the screening of the SNCA, UCHL-1 and LRRK2 genes. A total of 90 index cases with familial PD from Southern Italy. Moreover 100 healthy controls with a negative family history from Southern Italy were used in our study. Genomic DNA was extracted from peripheral blood using standard protocols. The eight exons (2, 4, 8, 9, 11, 14 25, 26) of GIGYF2, where the mutations have been found, are PCR amplified and sequenced. 90 index cases with familial PD have resulted negative at the mutations of SCNA, UCHL-1 and LRRK2 genes. Of these 90 we have already analyzed the eight exons of GIGYF2 on 50 patients. Among 50 patients screened, 2 carried an heterozygous mutation (c.3666G-A) in intron 26, 1 had a heterozygous synonymous mutation (Gln1215) in exon 25. These mutations were absent in controls. Furthermore we found two deletions and an insertion in exon 25 (Del LPQQQQQ 1209-1215, Del Q 1216, Ins Q 1217), with similar frequencies in PD cases and controls. Our future goal will be to continue the mutational analysis both in the remaining patients and in the other exons to look for novel mutations in the GIGYF2 gene.

#### P17.62

#### Rapid-onset dystonia parkinsonism (DYT12) and dysfunction of Na<sup>+</sup>/K<sup>+</sup>-ATPase Na<sup>+</sup> transport caused by a novel mutation in ATP1A3

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ATP1A3 encodes the alpha3 isoform of the Na<sup>+</sup>/K<sup>+</sup>-ATPase pump. Missense mutations in this gene have been reported to underlie rapid-onset dystonia parkinsonism (RDP, DYT12), but the physiological processes altered in this disease are not well understood. The Na<sup>+</sup>/K<sup>+</sup>-ATPase pump is a protein complex that exchange Na<sup>+</sup> and K<sup>+</sup> ions across the plasma membrane coupled to ATP hydrolysis, playing a fundamental role in maintaining the electrochemical gradient essential for neuronal function. The alpha subunit contains the catalytic site for ATP hydrolysis as well as the sites for binding and translocation of the ions. Recently, the first X-ray crystal structure of a Na<sup>+</sup>/K<sup>+</sup>-ATPase was reported for the pig renal enzyme.

We identified a new mutation in a patient with RDP, consisting of a tyrosine insertion at the very C-terminus of ATP1A3 (p.1013Ydup). This mutation was not found either in her healthy parents or in 218 controls. Ouabain viability assays indicated a drastic cell survival reduction, suggesting impaired pump function consistent with haploinsufficiency. Confocal scanning and Western blot studies supported that the altered pump function is not related to biogenesis, protein stability or plasma membrane targeting. Affinity studies in COS cells revealed a striking

40-50 fold reduction in  $\text{Na}^+$  affinity. The structural basis for this impairment in  $\text{Na}^+$  binding are provided by molecular modelling of ATP1A3 in the E1 ( $\text{Na}^+$ bound) conformation. Both the clinical presentation and the biochemical findings associated with the p.1013Ydup mutation provide *in vivo* and *in vitro* evidence for a crucial role of  $\text{Na}^+$  affinity in the pathophysiology of DYT12.

#### P17.63

##### A novel *SCN1A* mutation in a family with GEFS+ and sudden unexpected death in epilepsy (SUDEP)

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Sudden unexpected death in epilepsy (SUDEP) is a rare and unexplained cause of death in patients with epilepsy. Different risk factors and mechanisms may lead to a final common pathway of cardio-respiratory compromise. Mutations in the gene coding for the alfa-1 subunit of the neuronal voltage-gated sodium channel (*SCN1A*) gene, have been described in families with generalized epilepsy with febrile seizures plus (GEFS+). Mutations in genes coding for ion channels have also been associated to cardiac channelopathies. SUDEP is the most important direct epilepsy-related cause of death, and it has been suggested that some of these deaths may be due to cardiac arrhythmias. The neuronal voltage-gated sodium channel may have a role in pacemaker function of the sino-atrial node and blocking this channel slows the heart and increases heart rate variability.

The *SCN1A* gene was analyzed in a three generation GEFS+ family with two SUDEP cases. In this family, different epileptic phenotypes were present: Dravet syndrome, Doose syndrome and febrile seizures plus. Bi-directional sequencing of *SCN1A* revealed a new mutation (M1427T) in the patient with Dravet syndrome. This mutation is located in domain III, between transmembrane segments S5 and S6. Segregation analysis in the family revealed that all living members affected with epilepsy carried the mutation.

Our findings suggest that *SCN1A* mutations may play a role, either directly or indirectly, in SUDEP.

#### P17.64

##### Phenotypic variability of neuropsychiatric symptoms in EPM1-Unverricht-Lundborg disease (ULD)

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Purpose: To present the differences in neuropsychiatric symptoms in two unrelated individuals and two siblings, all with molecularly confirmed ULD.

Method: Review of medical records and investigations.

Results: Our four Caucasian patients had disease onset at the age of 8-10 years. They received valproic acid and various add-on medications. Patient 4 had a vagal nerve stimulator implanted.

Patient 1, a 30-year-old woman, has mild motor disability and mild mental retardation, adjustment disorder, drug abuse, and has had several suicide attempts.

Patient 2, her 31-year-old brother, walks independently, drinks alcohol since it has an antimyoclonic effect, has a history of drug abuse, and mood disorder.

Patient 3, a 25-year-old man, is able to walk and perform some activities of daily living, but presents frequent drop attacks. He has a positive mood, no psychiatric symptoms.

Patient 4, a 43-year-old man, is wheelchair bound. He is irritable and presents suicidal ideas, although these probably represent "acting-out" behaviour.

DNA analysis of the cystatin B gene showed: in patients 1 and 2, a dodecamer repeat expansion on one allele and a c.67-1G>C mutation on the other allele; in patient 3, a dodecamer repeat expansion on one allele and an IVS1-1G>C mutation on the other allele; in patient

4 (non-consanguineous parents), a dodecamer repeat expansion on both alleles.

Conclusion: In these patients, the differences in symptomatology and disability were striking and unexplained. Although the severity and rate of progression of ULD are known to be variable, the relationship to the type of mutation requires further study.

#### P17.65

##### Influence of polymorphism IVS5-91G>A in *SCN1A* gene in patients with partial epilepsy treated by carbamazepine monotherapy

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Introduction: Carbamazepine is still widely used as a first-line drug to treat partial epilepsy. It acts by binding to the  $\alpha$ -subunit of voltage-sensitive sodium channels in neurons. Despite its efficacy has been clearly proven, some discrepancies were observed in the treatment response with 30% of non-responders patients. The aim of this study is to determine whether nine functional single-nucleotide polymorphisms in the *SCN1A*, *SCN2A* and *SCN1B* genes, coding for ion sodium channels, correlate with epilepsy susceptibility and with response to carbamazepine.

Patients and methods: The population study included a French cohort of 47 patients with partial epilepsy treated by carbamazepine monotherapy and 95 healthy controls. Allele and genotype frequencies were compared between patients and controls, according to their response to carbamazepine therapy. The patients were considered to be drug responsive if they had not experienced any seizure for one year after receiving carbamazepine monotherapy. We also observed clinical correlation with carbamazepine maximum doses. Genotypes were determined on genomic DNA extracted from whole blood, using primer extension method (SnapShot, ABI).

Results: No association was found between these nine SNPs and epilepsy susceptibility. A significant correlation was found between one SNP (IVS5-91G/A) and response to treatment. A allele carriers get a better response to carbamazepine therapy. These results show a trend opposite to that described in previous studies. Larger studies are needed to confirm the relevance of this association.

#### P17.66

##### Study of the possible role of *DRD2* and *DAT1* gene polymorphisms on behavioral characteristics in animal model of epilepsy

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Despite of the increasing interest in the study of epilepsy known to be one of the severe psychoneurological disorders, mechanisms of its forming remain unclear. WAG/Rij strain rats belong to inbred line with genetically determined absens epilepsy and thus might be considered as model of epilepsy manifesting as result of sound sensitivity in particular. It has been reported earlier that dopaminergic system functioning is associated with epilepsy.

We aimed to define a single genotype effect of polymorphisms in dopaminergic system genes: *DRD2* rs8154872 in exon 7 and *DAT1* rs13448119 in exon 2 - on audiogenic sensitivity and on behavioral characteristics in WAG/Rij strain rats.

The present study sample was comprised of 55 WAG/Rij rats genotyped previously for *DRD2* Taq1A polymorphism in order to reveal inbred lines in direction to A1-allele and A2-allele. Audiogenic sensitivity was assessed by the presence / absence of epileptic seizure as response to stimuli, while exploratory and locomotor activities were detected by means of open-field test and elevated plus maze. Genotyping of two polymorphisms was performed using PCR, PCR-RFLP technique.

According to genotyping of *DAT1* rs13448119 polymorphism only samples with T/T genotype were detected, while analyzing *DRD2* rs8154872 polymorphism one C/T genotype was observed. Unfortu-

nately, revealed results did not allow performing statistical analyses. Since information involving SNPs heterozygosity in rats remains mainly unknown, following experiments based on other polymorphic loci in DRD2 and DAT1 genes are required.

#### P17.67

#### Febrile Seizure or Darvel Syndrome: A clinical and molecular study of SCN1A-related epilepsy in Iranian families

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Introduction: SCN1A-related seizure disorders encompass a spectrum that ranges from simple febrile seizures (FS) and generalized epilepsy with febrile seizures plus (GEFS+) at the mild end to Dravet syndrome at the severe end. The phenotype can vary even within the same family. Probands with autosomal dominant SCN1A-related seizure may have a de novo mutation. Most SCN1A-related SMEI are the result of a de novo heterozygous mutation. Early diagnosis prenatal diagnosis for pregnancies at increased risk is possible if the disease causing mutation in the family is known.

Material and methods: Classification of patients based on clinical examination and genetic counseling were done. DNA was obtained from 34 unrelated families with a spectrum of Idiopathic Epilepsy (IE). In order to finding point mutations and SNPs, exons of SCN1A, SCN1B and Mitochondrial common deletions, tRNA Lys tRNA Leu in probands were screened for any alteration using intronic primers and were analyzed by Single Strand conformation Polymorphism gel electrophoresis (SSCP) and any variation confirmed by direct sequencing. Allele and genotype frequencies in the patients and in the control groups were compared by  $\chi^2$  analysis, Fisher's exact test and logistic regression analysis methods.

Conclusions: We could found new intronic and exonic variants in candidate genes, in Iranian patients with IE subtypes. The high rate of heterogeneity and consanguinity, large families with many affected members in family as a social and healthy fact in Iranian populations could be a golden gene pool for linkage analysis in common disease.

#### P17.68

#### Role of SCN1B mutations in drug resistant in Iranian epileptic families

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Introduction: Many antiepileptic drugs (AEDs) prevent seizures by blocking voltage-gated brain sodium channels such as SCN1A, SCN2A, and SCN1B. However, treatment is ineffective in 30% of epilepsy patients, which might, at least in part, result from polymorphisms of the sodium channel genes. The R85C and R85H mutations of the  $\beta$ 1 subunit cause generalized epilepsy syndromes in humans and cause an increase in excitability but the R85H mutation was more excitable. We investigated the Role of SCN1B mutations in drug resistant in Iranian epileptic families.

Material and methods: Diagnostic classification of patients followed the proposal of the Commission on Classification and Terminology of the International League Against Epilepsy (1989). Family History, Electroencephalography (EEG) recordings and CT Scan were obtained from most patients. Written consent was obtained from all participants. DNA was obtained from 34 unrelated families with idiopathic generalized epilepsy as index families. The all coding exons of SCN1B were screened for deletions and duplications by MLPA. In order to finding point mutations and SNPs each exon individually amplified from genomic DNA in PCR reactions using intronic primers and were analyzed by Single Strand conformation Polymorphism gel electrophoresis (SSCP) and conformation-sensitive gel electrophoresis (CSGE) and so the PCR products with mobility variants were sequenced by ABI sequencer. Allele and genotype frequencies in the patients and in the

control groups were compared by either  $\chi^2$  analysis or Fisher's exact test.

Results: We have identified some new intronic variants in SCN1B and new mutations too, in patients with IE subtypes.

#### P17.69

#### Influence of SCN1A, SCN2A and SYN2 gene polymorphisms in epilepsy susceptibility and therapeutic efficacy

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Epilepsy is a common multifactorial neurological disorder, with higher prevalence in developing countries like India. Genetic variants of neuronal sodium channels; like SCN1A, SCN2A, SCN3A and other neuronal genes such as SYN2 have been implicated for their contribution in epilepsy susceptibility and its therapy. To evaluate sodium channel genes and synapsin vesicle associated gene SYN2 as candidates for the epilepsy susceptibility and their role in therapeutic efficacy, we screened two coding Single-nucleotide polymorphism of SCN1A p.T1056A (rs2298771), SCN2A 56G>A (rs17183814) and rs3773364 A>G intronic polymorphism in SYN2 gene in north Indian epilepsy patients. A total of 372 patients with epilepsy and 199 control individuals were enrolled for the study. The genotyping was performed using PCR-RFLP assay in all individuals. Therapeutic drug monitoring for phenytoin, carbamazepine, phenobarbital, and valproate was also performed in 20% of the patients to confirm compliance. Among all 372 patients with epilepsy, 118 were drug resistant and 254, were drug responsive. AG genotype of SCN1A 3184 A>G polymorphism was significantly higher and associated in epilepsy patients ( $P=0.005$ , OR = 1.764, 95% CI = 1.192-2.611) while G variant of SCN2A was associated with multiple drug resistance in north Indian patients with epilepsy ( $P=0.037$ ; OR 1.625 95% CI=1.030-2.564). The AG genotype of SYN2 gene also was significantly higher in patients with epilepsy versus control subjects in north Indian population ( $P=0.02$ , OR = 1.55, 95% CI = 1.066-2.269). Overall results indicate these genes and their variants have significant role in epilepsy susceptibility and could modulate drug response behavior as well.

#### P17.70

#### Two novel KCNQ2 mutations in Bulgarian patients with idiopathic neonatal epilepsy

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<sup>1</sup>National Genetics Laboratory, Sofia, Bulgaria, <sup>2</sup>Molecular Medicine Center, Medical University, Sofia, Bulgaria, <sup>3</sup>Department of Biochemistry, Tel Aviv University, Tel Aviv, Israel, <sup>4</sup>Department of Pediatrics, Medical University, Sofia, Bulgaria, <sup>5</sup>Department of Pediatrics, Medical University, Varna, Bulgaria, <sup>6</sup>VIB Department of Molecular Genetics, University of Antwerp, Antwerp, Belgium, <sup>7</sup>Department of Chemistry and Biochemistry, Medical University, Sofia, Bulgaria. Mutations in the KCNQ2 gene, encoding the voltage-gated potassium channel Kv7.2, have been identified as the underlying cause mainly for benign familial neonatal convulsions (BFNC). They are located predominantly in the pore region or the C-terminus of the Kv7.2 protein. We performed a point mutation screening of KCNQ2 in patients with idiopathic neonatal epilepsy, which included PCR analysis followed by direct sequencing of all exons and exon-intron boundaries of the gene. In addition, to determine the probable pathological effect of the mutations we elaborated a computational model of the Kv7.2 channel structure, using homology modeling. The predicted structure was based on the known 3D structure of Kv1.2-Kv2.1 chimera.

In this study, one novel de novo missense mutation and one novel familial nonsense mutation in KCNQ2 were found. The missense mutation is located in the pore region of the Kv7.2 and disrupts the whole channel structure. The mutation is arisen de novo in a girl with severe intractable epilepsy and developmental delay. The nonsense mutation is situated in the C-terminus of the channel and is predicted to cause protein truncation. It was found in two sisters with BFNC and their father.

This is the first KCNQ2 study in Bulgarian epilepsy patients. Although mutations in KCNQ2 are mostly considered to cause BFNC with AD inheritance, we show that in rare cases they can contribute to the pathogenesis of sporadic forms of severe neonatal epilepsy.

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**P17.71****Genetic screening of two Tunisian families with Generalized Epilepsy with febrile seizures plus (GEFS+)**

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Febrile Seizure can be associated with heterogeneous epilepsy phenotypes regrouped in a syndrome called generalized epilepsy with febrile seizures plus (GEFS+). The aim of this report is to search for the gene responsible for GEFS+ in two affected Tunisian families. The micro-

satellite marker analysis was performed on the known FS and GEFS+ loci. According to the results obtained by statistical analyses, GABRG2 on GEFS+3 locus and SCN1A on GEFS+2 locus were considered as two of the potential candidate genes and were tested for mutations by direct sequencing. The mutation analysis and statistical test of the GABRG2 gene revealed a disease association with rs211014 in intron 8 ( $\chi^2= 5.25$ ,  $P= 0.021$ ). A sequencing analysis of the SCN1A gene was performed for the two tested families and showed a known mutation (c.1811G>A) and a putative disease-associated haplotype in only one family. Our results support that SCN1A is the responsible gene for GEFS+ in one of the two studied Tunisian families and suggest a positive association of an intronic SNP in the GABRG2 gene in both families.

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